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President Truman signing the executive order transferring the U. S. Atomic Energy Program from military to civilian control.

Carroll L. Wilson, who was shortly before made general manager of the Atomic Energy Commission, is seated to the left, and David Lilienthal, chairman of the Commission, is seated on the right; standing, left to right, Sumner T. Pike, a member of the Commission; Col. K. D. Nichols, deputy chief of the Manhattan Engineer District; Secretary of War Robert Patterson; Maj. Gen. Leslie Groves, formerly in charge of the Manhattan Engineer District; Lewis L. Strauss and William W. Waymack, members of the Commission.

The Commission met in Washington with its advisory committee during the first week in January preparatory to taking over active control of more than 43,000 employees involved in the Manhattan Project.

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Theory of the Chain-reacting Pile
Enrico Fermi

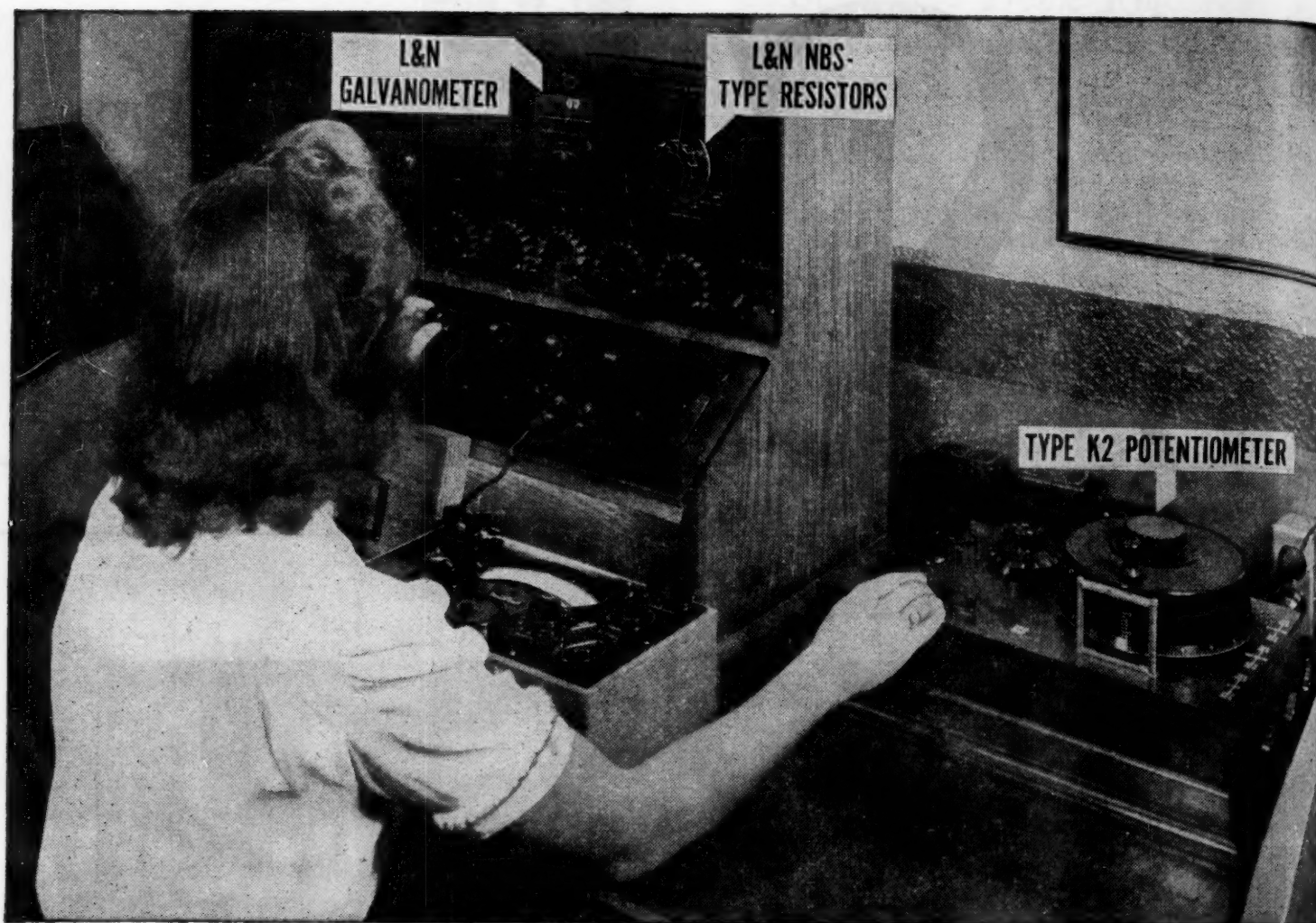
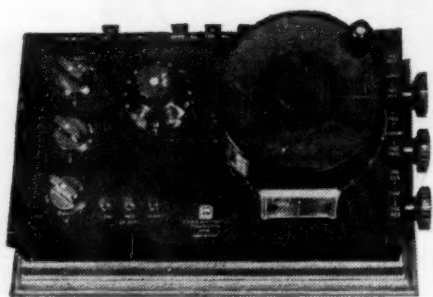


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Elementary Theory of the Chain-reacting Pile

Enrico Fermi

Institute for Nuclear Studies, University of Chicago

THE RESULTS AND THE METHODS DISCUSSED in the following outline of the theory of a chain-reacting pile working with natural uranium and graphite have been obtained partly independently and partly in collaboration by many people who participated in the early development work on the chain reaction. Very important contributions to the theoretical ideas were given by Szilard and Wigner. Many physicists contributed experimental results that helped to lead the way, among them, H. L. Anderson and W. H. Zinn, first at Columbia University and later at the Metallurgical Laboratory of the University of Chicago; R. R. Wilson and E. Creutz, at Princeton; and Allison, Whitaker, and V. C. Wilson, at the University of Chicago. The production of the chain reaction was finally achieved in the Metallurgical Laboratory directed by A. H. Compton.

ABSORPTION AND PRODUCTION OF NEUTRONS IN A PILE

We consider a mass, "the pile," containing uranium spread in some suitable arrangement throughout a block of graphite. Whenever a fission takes place in this system, an average number (ν) of neutrons is emitted with a continuous distribution of energy of the order of magnitude of 1,000,000 EV. After a neutron is emitted, its energy decreases by elastic collisions with the atoms of carbon and to some extent also by inelastic collisions with the uranium atoms. In the majority of cases the neutrons will be slowed down to thermal energies. This process requires about 100 collisions with carbon atoms. After the energy of the neutron is reduced to thermal value, the neutron keeps on diffusing until it is finally absorbed. In several cases, however, it will happen that the neutron is absorbed before the slowing-down process is completed.

The neutron may be absorbed by either the carbon or the uranium. The absorption cross-section of carbon for neutrons of thermal energy is quite small, its value being approximately $.005 \times 10^{-24}$ cm.² For graphite of density 1.6, this corresponds to a mean free path for absorption of about 25 m. It is believed that the absorption cross-section follows the $1/v$ law, and consequently the absorption cross-section, which is already quite small at thermal energies, becomes practically negligible for neutrons of higher energy. It is therefore a sufficiently

This paper, presented at the Chicago meeting of the American Physical Society, June 21, 1946, is based on work performed under Contract No. W-7401-eng-37 with the Manhattan District at the Metallurgical Laboratory, University of Chicago.

good approximation to assume that absorption by carbon during the slowing-down process can be neglected.

The absorption of a neutron by uranium may lead either to fission or to absorption by a (n, γ) process. We shall refer to this last possibility as the process of resonance absorption. The relative importance of fission and resonance absorption in the different energy intervals is not the same. In this respect we can consider roughly three intervals:

(1) Neutrons with energy above the fission threshold of U^{238} —We can call these conventionally "fast neutrons." For fast neutrons the most important absorption process is fission, which normally takes place in the abundant isotope U^{238} . Resonance absorption is smaller but not negligible.

(2) Neutrons of energy below the fission threshold of U^{238} and above thermal energy—We shall refer to these neutrons as "epithermal neutrons." For epithermal neutrons the most important absorption process is the resonance capture. The cross-section for this process as a function of energy is quite irregular and presents a large number of resonance maxima that can be fairly well represented by the Breit-Wigner theory. In practical cases the resonance absorption becomes important for neutron energy below about 10,000 EV and increases as the energy of the neutrons decreases.

(3) Neutrons having thermal agitation energy or "thermal neutrons"—For thermal neutrons both the resonance and fission absorption processes are important. In this energy range both cross-sections follow approximately the $1/v$ law, and therefore their relative importance becomes practically independent of the energy. Let σ_f and σ_r be the cross-sections for fission and resonance absorption for neutrons of energy kT , and η be the average number of neutrons emitted when a thermal neutron is absorbed by uranium. Then η differs from ν , since only the fraction $\sigma_f/(\sigma_r + \sigma_f)$ of all the thermal neutrons absorbed by uranium produces a fission. It is, therefore,

$$\eta = \nu\sigma_f/(\sigma_r + \sigma_f). \quad (1)$$

The preceding discussion leads one to conclude that only a fraction of the original fast neutrons produced will end up by producing a fission process. For systems of finite size, further losses of neutrons will be expected by leakage outside the pile.

Limiting ourselves for the present to systems of practically infinite dimensions, we shall call P the probability that a fast neutron ultimately is absorbed by the fission

process. The average number of neutrons produced in the "second generation" by the first neutron will then be

$$k = P\nu. \quad (2)$$

Usually, k is called the "reproduction factor" of the system. A self-sustaining chain reaction evidently is possible only when $k > 1$. If this is the case, the reaction actually will take place provided the leakage loss of neutrons is sufficiently small. This, of course, can always be achieved if the size of the pile is large enough.

LIFE HISTORY OF A NEUTRON

When a fast neutron is first emitted in our pile, the following events may take place:

(1) There is a small probability that the neutron will be absorbed by uranium before its energy has been appreciably decreased. If this is the case, the absorption leads often to fission of U^{235} . The probability of such fast fissions, however, is usually only a few per cent. Indeed, if the system contains little uranium and a large amount of carbon, the elastic collisions with carbon tend to reduce the energy very rapidly to a value below the fission threshold of U^{235} . If, on the other hand, the system is very rich in uranium, the inelastic collision processes become very probable and rapidly reduce the energy of the original fast neutron to a fairly low value before it has a chance to produce a fission in U^{235} .

(2) In the large majority of the cases, therefore, the neutron is not absorbed as a fast neutron and rapidly loses its energy, mostly due to collision against the carbon atoms. One can prove in an elementary way that it takes about 6.3 collisions against the carbon atoms to reduce the energy by an average factor of e . Consequently, it will take about 14.6 collisions in order to reduce the energy by a factor of 10, and about 110 collisions to reduce the energy from 1,000,000 EV to the thermal energy value of $1/40$ v. While this slowing-down process is in progress, the neutron may be absorbed by the resonance process in uranium. We shall call p the probability that a neutron is not absorbed before reaching thermal energy. One of the most important factors in designing a pile consists in trying to minimize the probability that neutrons are removed from the system by resonance absorption during the slowing down.

(3) If the neutron is not absorbed during the slowing-down process, it eventually reaches thermal energy and ultimately will be absorbed by either uranium or carbon. If uranium and carbon were mixed uniformly, the probability for these two events would be in the ratio of the absorption cross-sections of uranium and carbon for thermal neutrons multiplied by the atomic concentrations of the two elements. Since actually the mixture is not uniform, this is only approximately true. We shall call f the probability that a thermal neutron is absorbed by uranium. In designing a chain-reacting pile one will

normally try to adjust things so as to have both f and p as large as possible. Unfortunately, the two requirements are contradictory, because in order to make f large, one shall try to build a system very rich in uranium in order to reduce the probability of absorption of thermal neutrons by carbon. On the other hand, in a system containing a relatively small amount of carbon the slowing-down process will be relatively slow, and consequently the probability of resonance absorption during the slowing down will be large.

It is clear, therefore, that one shall have to conciliate two opposite requirements by finding an optimum value for the ratio of uranium to carbon.

In a homogeneous mixture of uranium and carbon the values of f and p depend only on the relative concentrations of the two elements. If we do not restrict ourselves, however, to homogeneous mixtures only, one can try to obtain a more favorable situation by proper arrangement of the geometrical distribution of the two components. This actually is possible to a considerable extent, because of the following circumstances. The resonance absorption which is responsible for the loss of neutrons during the slowing down has very sharp cross-section maxima of the Breit-Wigner type. Therefore, if the uranium, instead of being spread through the graphite mass, is concentrated in rather sizable lumps, we will expect that the uranium in the interior of a lump will be shielded by a thin surface layer from the action of neutrons with energy close to a resonance maximum. Therefore, the resonance absorption of a uranium atom inside the lump will be much less than it would be for an isolated atom. Of course, self-absorption in a lump reduces not only the resonance absorption but also the thermal absorption of uranium. One can expect theoretically, however, and experiment has confirmed, that at least up to a certain size of lumps the gain obtained by reducing the resonance loss of neutrons overbalances by a considerable amount the loss due to a lesser absorption of thermal neutrons.

The typical structure of a pile is a lattice of uranium lumps embedded in a matrix of graphite. The lattice may be, for example, a cubic lattice of lumps or a lattice of rods of uranium. This latter arrangement is slightly less efficient from the point of view of the neutron absorption balance but often presents some practical advantages, since it makes easier the removal of the heat produced by the pile. In the present discussion we shall consider only lattices of lumps.

It is useful to give some typical figures for the probabilities of the various absorption processes. These probabilities, of course, are not constant but depend on the details of the structure of the lattice. Average figures for a good lattice will be given as an example. When a neutron is first produced by a fission taking place in a lump of uranium, it may have a probability of the order of 3 per cent of being absorbed, giving rise to fission

before losing any appreciable amount of energy. In 97 per cent of the cases when this does not happen the neutron will initiate its slowing-down process, and it may either be absorbed by the resonance process during the slowing down or reach thermal energy. The probability of resonance absorption during the slowing down may be of the order of 10 per cent, so that 87 per cent of the original neutrons will be slowed down to thermal energies. Of these, perhaps 10 per cent may be absorbed by carbon and the remaining 77 per cent by uranium. If we assume for the purpose of example that $\nu = 2$, we shall have in one generation the processes summarized in Table 1. For the example given, the reproduction factor will be, therefore,

$$k = .06 + .77\eta. \quad (3)$$

Consequently, a lattice of the type described would have a reproduction factor larger than 1, provided η is larger than 1.22.

In order to evaluate the reproduction factor one must

TABLE 1

Probability (%)	Type of process	Neutrons produced per neutron absorbed	Neutrons per generation by one neutron
3	Fast fission	2	.06
10	Resonance absorption	0	0
10	Absorption by carbon	0	0
77	Absorption by uranium at thermal energies	η	.77 η

be able to calculate the probabilities for the various processes mentioned. Some points of view which may be used in the practical calculation will be indicated briefly.

PROBABILITY OF FISSION BEFORE SLOWING DOWN

The value of this quantity is very easily calculable for a very small lump of uranium. In this case it is obviously given by

$$P_F = \sigma_F n d, \quad (4)$$

where σ_F is the average value of the fission cross-section for fission neutrons; n is the concentration of uranium atoms in the lump; and d is the average value of the distance that the neutron produced in the lump must travel before reaching the surface of the lump. The case of a lump of larger size is more complicated, since then multiple collision processes become important and both elastic and inelastic scattering play a considerable role. In particular, the last process for a lump of large size effectively slows down the neutrons before the fission threshold of U^{235} and brings them down to an energy

level in which they are readily absorbed by the resonance process.

RESONANCE ABSORPTION

If we had a single atom of uranium in a graphite medium where fast neutrons are produced and slowed down to thermal energy, the probability per unit time of a resonance absorption process of neutrons with energy larger than thermal energy would be given by the following expression:

$$\frac{q\lambda}{.158} \int \sigma(E) \frac{dE}{E}, \quad (5)$$

where q is the number of fast neutrons entering the system per unit time and unit volume, λ is the mean free path, and $\sigma(E)$ is the resonance absorption cross-section at energy E . The integral must be taken between a low limit just above thermal energy and an upper limit equal to the average energy of the fission neutrons. One will expect that the largest contribution to the integral will be due to the Breit-Wigner peaks of $\sigma(E)$.

The above formula would be very much in error in the case of a lattice of lumps. As already indicated, this is due to the fact that inside a lump there is an important self-screening effect that reduces very considerably the density of neutrons having energy close to a resonance maximum.

The best approach to a practical solution to the problem is therefore a direct measurement of the number of neutrons absorbed by resonance in lumps of uranium of various sizes.

Measurements of this type have been performed first at Princeton University, and the results have been summarized in practical formulas that are used in the calculations.

PROBABILITY OF ABSORPTION AT THERMAL ENERGIES

If uranium and carbon were uniformly mixed, a thermal neutron would have a probability

$$\frac{N_U \sigma_U}{N_C \sigma_C + N_U \sigma_U} \quad (6)$$

to be absorbed by uranium. In this formula N_C and N_U represent the numbers of atoms of carbon and of uranium per unit volume, and σ_C and σ_U represent the cross-sections of carbon and uranium for thermal neutrons.

More complicated is the case of a lattice distribution of lumps of uranium in graphite, since the density of thermal neutrons throughout the system is not uniform but is large at the places far from the uranium lumps and smaller near and inside the uranium lumps, due to the fact that the absorption of thermal neutrons is much greater in uranium than in graphite. Let \bar{n}_C and \bar{n}_U be the average densities of thermal neutrons in the graphite

and in the uranium lumps. The number of thermal neutrons absorbed by uranium and by carbon will be proportional to $N_U \sigma_U \bar{n}_U$ and $N_C \sigma_C \bar{n}_C$, and we will have, therefore, instead of Equation (6), the corrected formula,

$$f = \frac{N_U \sigma_U \bar{n}_U}{N_U \sigma_U \bar{n}_U + N_C \sigma_C \bar{n}_C} \quad (7)$$

For practical purposes it is usually sufficiently accurate to calculate \bar{n}_C and \bar{n}_U , using the diffusion theory. The approximation is made to substitute the lattice cell by a spherical cell having volume equal to that of the actual cell, with the boundary condition that the radial derivative of the density of neutrons vanishes at the surface of the sphere. It is also assumed that the number of neutrons that are slowed down to thermal energies per unit time and unit volume is constant throughout the graphite part of the cell. This approximation is fairly correct, provided the dimensions of the cell are not too large. With these assumptions one finds the following formula for the probability, f , that thermal neutrons be absorbed by uranium:

$$f = \frac{3\alpha^2}{\alpha^3 - \beta^3} \frac{(1 - \alpha)(1 + \beta)e^{-\beta + \alpha} - (1 + \alpha)(1 - \beta)e^{\beta - \alpha}}{(\alpha + s - s\alpha)(1 + \beta)e^{-\beta + \alpha} - (\alpha + s + s\alpha)e^{\beta - \alpha}} \quad (8)$$

where α and β represent the radius of the lump and the radius of the cell expressed taking the diffusion length in graphite, $l = \sqrt{\lambda\Lambda/3}$, as unit of length. It is further

$$s = \frac{\lambda}{\sqrt{3}} \frac{1 + \gamma}{1 - \gamma} \quad (9)$$

where γ is the reflection coefficient of the lump for thermal neutrons.

LATTICE CONTAINING A LARGE NUMBER OF CELLS

The density of neutrons of any given energy in a lattice containing a large number of cells is a function of the position in the lattice. One can arrive at a simple mathematical description of the behavior of such a system by neglecting in first approximation the local variation of such functions due to the periodic structure of the lattice and substituting for the actually inhomogeneous system an equivalent homogeneous system. In this section we shall accordingly simplify the problem by substituting for all densities of neutrons values obtained by averaging the actual values over the volume of the cell. The densities will then be represented by smooth functions such as one would expect in a homogeneous uranium-graphite mixture.

Let $Q(x, y, z)$ be the number of fast neutrons produced per unit time and unit volume at each position in the

lattice. These neutrons diffuse through the mass and are slowed down. During this process some of the neutrons are absorbed at resonance. Let $q(x, y, z)$ be the number of neutrons per unit time and unit volume which become thermal at the position x, y, z — q is called the "density of the nascent thermal neutrons."

We shall assume that if an original fast neutron is generated at a point, 0, the probability that it becomes thermal at a given place has a Gaussian distribution around 0. This assumption may be justified by considering that the diffusion process of slowing down consists of very many free paths. Experimentally one finds that the distribution curve of the nascent thermal neutrons around a point source of fast neutrons is represented only approximately by a Gaussian distribution, and formulas have been used in which the actual distribution is described as a superposition of two or three Gaussian curves with different ranges. For the purpose of the present discussion, however, we shall take only one. For each fast neutron produced only p neutrons reach thermal energy. The distribution of nascent thermal neutrons produced by a source of strength 1, placed at the origin of the coordinate, shall then be represented by

$$q_1 = \frac{P}{\pi^{3/2} r_0^3} e^{-r^2/r_0^2} \quad (10)$$

For graphite of density 1.6 the range, r_0 , is of the order of 35 cm. The density of nascent thermal neutrons at point P can be expressed in terms of Q by adding up the contribution of all the infinitesimal sources, $Q(P')d\tau'$ ($d\tau'$ represents the volume element around the point, P'). We obtain in this way

$$q(P) = \frac{P}{\pi^{3/2} r_0^3} \int Q(P') e^{-((P'-P)^2/r_0^2)} d\tau' \quad (11)$$

The density, $n(x, y, z)$, of the thermal neutrons is connected to q by the differential equation,

$$\frac{\lambda v}{3} \Delta n - \frac{v}{\Lambda} n + q = 0, \quad (12)$$

where λ is the collision mean free path of thermal neutrons, v is their velocity, and Λ is the mean free path for absorption of a thermal neutron. Equation (12) is obtained by expressing a local balancing of all processes whereby the number of thermal neutrons at each place tends to increase or decrease. The first term represents the increase in number of neutrons due to diffusion ($\lambda v/3$ is the diffusion coefficient of thermal neutrons); the second, the loss of neutrons due to absorption; and the third, the effect of the nascent thermal neutrons.

It should be noted that the absorption mean free path Λ in Equation (12) is much shorter than the corresponding quantity, Λ_0 , in pure graphite. Indeed, the absorp-

tion in a lattice is due mostly to the uranium. In first approximation Λ is given by

$$\Lambda = (1 - f)\Lambda_0. \quad (13)$$

In practical cases Λ may be of the order of magnitude of 300 cm., whereas Λ_0 in graphite without uranium is about 2,500 cm.

When a thermal neutron is absorbed by uranium, η new neutrons are produced by fission. This number should be increased by a few per cent in order to take into account the effect of the small probability of fast fission. Let $\epsilon\eta$ be the total number of fast neutrons so corrected.

The number of thermal neutrons absorbed per unit volume and unit time is $\frac{vn}{\Lambda}$. Of these, the fraction f is absorbed by uranium. We have, therefore,

$$Q = f\eta \epsilon \frac{v}{\Lambda} n + Q_0, \quad (14)$$

where $f\eta \epsilon \frac{v}{\Lambda}$ represents the number of fast neutrons produced in the chain reaction process, and Q_0 represents the number of fast neutrons produced by an outside source if one is present. In most cases, of course, Q_0 will be equal to 0. From Equations (11), (12), and (14) we can eliminate all unknowns except n , and we find

$$\frac{3}{\Lambda\Lambda} n - \Delta n = \frac{3p \epsilon \eta f}{\pi^{3/2} r_0^3 \Delta \lambda} \int n(P') e^{-(P'-P)^2/r_0^2} d\tau' + \frac{3p}{\pi^{3/2} r_0^3 \lambda v} \int Q_0(P') e^{-(P'-P)^2/r_0^2} d\tau'. \quad (15)$$

A solution of this equation is obtained readily by developing both Q_0 and n in a Fourier series. The general term of this development, corresponding to Q_0 of the form $Q_0 \sin \omega_1 x \sin \omega_2 y \sin \omega_3 z$, is:

$$n = \frac{(\Delta p Q_0 / v) \sin \omega_1 x \sin \omega_2 y \sin \omega_3 z}{\left(1 + \frac{\lambda \Lambda}{3} \omega^2\right) e^{\omega^2 r_0^2/4} - \epsilon p f \eta}, \quad (16)$$

where $\omega^2 = \omega_1^2 + \omega_2^2 + \omega_3^2$.

When the dimensions of the pile are finite but very large compared with the mean free path, the boundary condition is that all densities must vanish at the surface. If the pile, for example, is a cube of side a and the origin of the coordinates is taken in one of the corners, it is:

$$\omega_1 = \frac{\pi n_1}{a}; \quad \omega_2 = \frac{\pi n_2}{a}; \quad \omega_3 = \frac{\pi n_3}{a}, \quad (17)$$

where n_1, n_2, n_3 are positive integral numbers that define the various Fourier components. The critical dimensions of the system are such that the denominator of

Equation (16) vanishes for the 1,1,1 harmonic, since in this case the density of the neutrons becomes infinitely large. The critical condition can be expressed, therefore, by the equation:

$$\left(1 + \frac{3\pi^2 \lambda \Lambda}{a^2} \frac{1}{3}\right) e^{3\pi^2/a^2 r_0^2/4} = \epsilon p f \eta. \quad (18)$$

The right-hand side in this formula is the reproduction factor, k , for a system of infinite size. We can therefore write the critical condition as follows:

$$k = \left(1 + \frac{3\pi^2 \lambda \Lambda}{a^2} \frac{1}{3}\right) e^{3\pi^2/a^2 r_0^2/4}. \quad (19)$$

In most cases both the exponent of e and the term added to 1 in the parentheses are small compared with 1, and so the previous expression can be simplified to:

$$k = 1 + \frac{3\pi^2}{a^2} \left(\frac{\lambda \Lambda}{3} + \frac{r_0^2}{4}\right). \quad (20)$$

This formula can be used in order to calculate the critical side of a pile of cubical shape. If, for example, we assume for a special lattice numerical values of $\lambda = 2.6$ cm., $\Lambda = 350$ cm., $r_0^2 = 1,200$ cm.², and $k = 1.06$, we find for the critical side of a cubical pile, $a = 584$ cm. Naturally, these constants are merely hypothetical, and though included within the possible range, are in practical cases strongly dependent on the details of the lattice structure.

It is useful to derive an approximate relationship between the power produced by a pile and the intensity of thermal neutrons inside it. Roughly 50 per cent of the thermal neutrons absorbed in a pile give rise to fission, and the energy released per fission is of the order of 200 MEV. This corresponds to about 1.6×10^{-4} ergs per thermal neutron absorbed. Since the number of thermal neutrons absorbed per unit volume is vn/Λ , the energy produced is approximately

$$\frac{vn}{\Lambda} 1.6 \times 10^{-4} \cong 4.6 \times 10^{-7} vn \text{ ergs/cm.}^3 \text{ sec.} \quad (21)$$

Naturally, the power is not produced uniformly throughout the pile because n is a maximum at the center and decreases to 0 at the edge of the pile. For a cubical pile n is represented approximately by

$$n = n_0 \sin \frac{\pi x}{a} \sin \frac{\pi y}{a} \sin \frac{\pi z}{a}, \quad (22)$$

where n_0 is the density of neutrons at the center of the pile. Integrating the previous expression (21) over all the volume of the pile, one obtains the following formula for the power:

$$W = \frac{8}{\pi^3} 4.6 \times 10^{-7} n_0 v a^3 = 1.2 \times 10^{-7} n_0 v a^3. \quad (23)$$

If, again, we take as an example a pile with a side of 584 cm., we find $W = 24 n_0 v$ ergs/sec. When the pile is operating at a power of 1 kw., the flux of thermal neutrons at the center is therefore about $n_0 v = 4 \times 10^8$ neutrons/cm.² sec.

DESCRIPTION OF A GRAPHITE PILE AT ARGONNE LABORATORY

The first pile was erected under the West Stands on the campus of the University of Chicago at the end of 1942. After having been operated there for a few months it was moved to the Argonne Laboratory, near Chicago, where it has been used until now for various research purposes.

The lattice of that pile is not the same throughout the structure. Since only a small amount of uranium metal was available at that time, metal has been used in the central portion of the pile and uranium oxide in the outer portion.

The intensity of operation of the pile is recorded by a number of BF_3 ionization chambers connected to amplifiers or to galvanometers.

Since this pile has no cooling devices built into it, the power produced is limited by the necessity of avoiding an excessive temperature rise. The pile could be operated indefinitely at a power of 2 kw. and is often operated for periods of the order of one or two hours up to about 100 kw.

One feature that is often used for neutron research work is the thermal column, a column of graphite having sides of about 5 x 5 feet, which is built on the center of the top of the pile and goes through the top shield. The neutrons that diffuse from the pile into this column are rapidly reduced to thermal energy so that the neutrons inside the column a few feet above the top of the pile are practically pure thermal neutrons.

The pile is also equipped with a number of holes in the shield and removable stringers of graphite that make it possible to explore phenomena inside the pile or to introduce samples for neutron irradiation.

When the pile is operated at 100 kw., the flux of thermal neutrons at the center is about 4×10^{10} neutrons/cm.² sec.

Work With Residual DDT Spray in Puerto Rico:

A Report of the First Year's Work

Porter A. Stephens and Harry D. Pratt
U. S. Public Health Service, Atlanta, Georgia

IN THE FALL OF 1944, the U. S. Public Health Service, in cooperation with the Insular Health Department and the School of Tropical Medicine, inaugurated a residual DDT spray project to determine whether this new method of malaria control which has been so remarkably successful in other parts of the world might be of practical value in Puerto Rico, where the important vector (*Anopheles albimanus*) is a "wild" mosquito which feeds on humans during twilight or at night and seldom remains inside houses for more than a few hours. The results of the first year's work are presented herein.¹

Two villages approximately 30 miles apart and rather similar in size, population, racial composition, occupa-

¹ The studies on which this report are based were conducted jointly by personnel of the U. S. Public Health Service District #6, Communicable Disease Center activities; the Bureau of Malaria Control, Puerto Rico Department of Public Health; and the School of Tropical Medicine, San Juan, Puerto Rico. The writers wish to acknowledge the assistance and cooperation of officials of the Communicable Disease Center, Atlanta, Georgia; Carter Memorial Laboratory, Savannah, Georgia; Office of Malaria Investigations, National Institute of Health; and District #6, U. S. Public Health Service. The commissioner, Insular Health Department, and the director of the School of Tropical Medicine also have given valuable advice and assistance. Army officials at Fort Bundy generously provided a number of buildings for experimental use.

tion of inhabitants, house construction, rainfall, general ecology, relation to vector-breeding areas, proximity to ocean, relation to rivers, isolation, and mosquito populations were selected for the experiment. Humacao Playa, on the east coast, was chosen as the test village to be sprayed with DDT, while Loiza Aldea, on the northeast coast, was used as an untreated check village.

In each village *A. albimanus* indices were obtained by animal-bait and light trap collections throughout the year. The only previous, long-range malaria control experiment in Puerto Rico was conducted at Salinas during the period 1930-36 and has been reported by Earle (1). His data show that in this small, unscreened native village little reduction in number of malaria cases occurred until the *albimanus* population had been reduced to such a low level that animal-bait traps collected less than one *albimanus* per night. In the villages used in the present experiment, bait and light traps caught from one to several hundred *albimanus* on most nights when traps were operated. It is therefore believed that *albimanus* was present in sufficient numbers to transmit malaria throughout the study period.

Unlike the DDT residual spray work with *A. quadripunctatus* at Stuttgart, Arkansas, and with *A. pseudoscorpionis*

punctipennis in Mexico, reported by Stage (2), no reduction in numbers of *A. albimanus* was found at either Puerto Rican town. In fact, collections of *A. albimanus* at Humacao Playa were actually larger in October and November 1945 than those made in October and November 1944 from identical bait and light traps in the same locations, even though the village had been treated with residual DDT spraying twice between November 1944 and October 1945.

Normally, in Puerto Rico the peak incidence of *A. albimanus* is reached in the fall and winter months from September through December or January, depending on

TABLE 1
COMPARISON OF MALARIA RATES IN UNTREATED AND TREATED VILLAGES
IN PUERTO RICO

Date	Total number of positive films of:				Total films			Positive films (%)
	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. under-term.</i>	Positive	Negative	All	
(Untreated Village—Loiza Aldea)								
First survey November 1-15, 1944	26	30	1	3	60	1,210	1,270	4.7
Second survey March 19-April 5, 1945	7	6	0	0	13	830	843	1.5
Third survey November 1-15, 1945	13	20	0	0	33	835	868	3.8
(Treated Village—Humacao Playa)								
First survey October 15-31, 1944	47	33	2	3	85	1,384	1,469	5.8
First residual DDT spraying November 1-17, 1944								
Second survey March 5-14, 1945	9	19	1	5	34	1,177	1,211	2.8
Second residual DDT spraying June 15-29, 1945								
Third survey October 15-31, 1945	5	4	1	0	10	1,083	1,093	0.91

the distribution of rainfall during this period, which constitutes the "rainy season." In most areas where malaria surveys have been conducted over a period of years it has been found that the greatest number of malaria cases occur during this same period, usually lagging a month or two behind the peak in *albimanus* population. Then there follows a marked reduction in the number of malarial mosquitoes and a gradual decline in number of cases of malaria during the period from January to, or through, April, which constitutes the "dry season." Depending upon the beginning of the "rainy season," malarial mosquito production and number of malaria cases begin increasing in late April or early May and continue at an accelerated rate until the period October to January, when the "dry season" begins again.

During the course of this study, three blood film surveys were made: the first, in October and November 1944;

the second, in March and April 1945; and the third, in October and November 1945. The slides were stained within 24 hours of the time of collection, and later sent to the Memphis or Atlanta laboratories of the U. S. Public Health Service for examination by trained technicians. The results are given in Table 1.

It will be seen from examination of Table 1 that the percentage of positive malaria slides from the untreated check village of Loiza Aldea followed approximately the normal cycle for Puerto Rico, with 4.7 per cent positive during the "rainy season" of November 1944, a decrease to 1.5 per cent positive during the "dry season" of March-April 1945, and a marked increase to 3.8 per cent positive during the "rainy season" of November 1945. On the other hand, in the village of Humacao Playa, which was sprayed with DDT twice during the period November 1944 to October 1945, there was a progressive decline in percentage of positive malaria slides: from 5.8 per cent in the "rainy season" of October 1944 to 2.8 per cent in the "dry season" of March 1945; and most significantly of all, to 0.91 per cent during the "rainy season" of October 1945.

It is believed that these data, which show a marked reduction in malaria in the DDT-treated village, indicate that DDT may be an effective weapon against malaria in the areas where the vector mosquito is of the "wild" variety, such as *A. albimanus*. Further experience will be necessary before its effectiveness under other conditions and against species with similar habits can be evaluated.

Details of DDT residual spray procedure will be discussed in a subsequent paper. However, it may be stated that approximately 500 houses with adjacent out-buildings were treated three times during the first year: the first residual spraying in November 1944, with an over-all average of 309 mg. of DDT per square foot, at a cost of \$1.31 per house; the second, in June 1945, with an average of 147 mg. per square foot, at a cost of \$.98 per house; and the third, in June 1945, with an average of 190 mg. per square foot, at a cost of \$.98 per house.

Puerto Rican families average about five persons each. Thus, the per capita cost was between \$.20 and \$.26 for a single application of DDT in Humacao Playa, or between \$.60 and \$.80 a year. This is not an exorbitant figure when it is realized that, in addition to reducing malaria, residual DDT spraying may play an important part in preventing the transmission of other insect-borne diseases of the Island, such as filariasis and dengue, and other diseases such as typhoid fever, diarrhea, and dysentery in which flies may play a vector role. Moreover, residual DDT spraying is effective in controlling such common household pests as bedbugs and cockroaches for several weeks or months.

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Research or Available Knowledge:

A Matter of Classification

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THE SCIENTIFIC DEVELOPMENTS of the past five years are fabulous by previous standards. Some are widely known. Radar and the atomic bomb, for example, have become household words. Many of the less spectacular developments are no less important, even though known and understood only by those few people who have taken part in their evolution and application to practical purposes. Impressive and vast as are the projects in which these accomplishments have been made, they actually consist of countless thousands of individual details of scientific progress. In the majority of cases these details have been worked out as a result of long and painstaking efforts and a great deal of "research."

The term "research" has gained the respect and even the awe of our people because they are shown results on an impressive scale flowing out of large-scale efforts described as "research." But the condition of the sources of information which must be searched by research workers is chaotic indeed. Two and a half million different U. S. Patents stand as one example of the number of items that can be involved in dealing with technical information, not to mention the many additional millions of foreign patents. Scientific papers and dissertations in this and other countries add a formidable number of possible sources of detailed information and guidance. As long ago as 1933, S. C. Bradford, of the Science Museum Library in London, estimated that 750,000 scientific and technical papers were published annually in 14,000 different periodicals. More recent estimates indicate that the rate has doubled since then.

In the early days of science, when it was much less complex and confusing, philosophers discoursed on how it grew. Progress, they discovered, came most effectively when that which was already known was fully organized and sorted out. Generalities on which future advances could be based were made only by a careful analysis of the pattern formed by that which was already known. This was obvious to Aristotle 2,300 years ago and was expounded in his *Posterior analytics*. Today science has acquired so many facets, so many millions of possible channels of development, that it is far more difficult to organize into a coherent pattern. Our libraries, universities, research laboratories, learned societies, and industrial organizations all struggle in futile fashion with their individual segments of the problem.

The obvious need is for the coordination and standardization of the system by which all such material is sorted and classified, so that all who may be interested

can at least direct their attention to a certain small portion of the whole mass of published material, selected in terms which are defined alike for all. Some organization, either public or private, must accept the burden of taking the infinite pains necessary to see that all scientific subject matter is classified according to logical principles, on a standardized basis acceptable to all who need it, whether for future scientific development or for the application of that development to industrial production.

Libraries classify their books and periodicals according to a number of systems, two of which are the most widely recognized. In this country many libraries follow a system established by the Library of Congress, using an arbitrary combination of letters and numerals. Many others, both here and abroad, follow a generally standard decimal system. This latter has great possibilities in providing a precise diagnosis, especially when the number of decimal places used grows to 12 or 15 digits. Unfortunately, much of the expansion has been made by people whose interest was in sorting and finding material for strictly literary purposes.

The Patent Office, faced with a growing mountain of patents to be issued, has been forced to make some progress toward a universal classification system as applied to technical subjects. The millions of patents are now grouped in some 300 main classes, which are further divided into over 40,000 specific subclasses. Each subclass contains a particular field of inventions, with an explicit definition. This definition is carefully worked out to indicate material excluded from the particular subclass defined, with guiding search notes to help find such material. Obviously, the subclasses themselves are grouped to facilitate the selection of the minimum field of search necessary to assure finding the particular subject matter required. Necessity has also brought into existence in the Patent Office a small staff of experienced workers constantly studying the need for additional subdivisions or arrangements to provide for growing arts.

The system used in the classification of patents has been dictated by the needs of those who must use it constantly in their work, in examining patent applications for novelty and in making searches to determine whether a given patent is completely valid and infringed by the manufacture or sale of some device or the use of a certain process. The controlling factor in classifying patents must be the most generic or essential function of the device classified. An internal combustion engine, for example, is a device for producing power, whether that power drives an automobile or pumps water. Classifying the engine under automobile engines would effectively

lose it for anyone seeking the details of an exactly similar engine which he had conceived as part of a system for pumping water.

In patent searching it is necessary to be certain that the search can be absolutely complete after looking at as few disclosures as possible. One patent erroneously classified and not located may turn up later and destroy a business founded on the studied belief that no patent was infringed or that the patents on which it was founded were completely new and valid. Yet there is a practical limit to the searching possible to determine those facts. However detailed a library type of classification may become, it is not concerned with the problem of limiting the number of items placed in a given subclass.

Many large corporations have collected the few thousands of patents in fields of special importance to them and have then sorted them still further according to systems which happen to meet their particular requirements. In some cases the same system is then used to some extent to sort and classify collections of published articles and other available material in the selected field, and frequently is used as a guide to research programs. The problem of handling published material faces all organizations engaged in scientific and technical research. There are countless librarians and clerks in research laboratories all over the country, patiently making card files and cross-indexes of journal articles and other material that has been found useful or for which a future need is anticipated. Some few of these collections have been further organized to permit the use of mechanical devices such, for example, as punch-card collections and sorting machines to select cards that may represent precise groups of patents or published articles pertaining to a particularly defined problem.

There are machines now available which can select such things as cards or films according to codifications of an almost infinite number of subdivisions. The card or film may also carry microfilm or microprint reproductions of the material it classifies. The interest in this type of equipment was illustrated recently during the symposium of the American Chemical Society at Chicago. A day was devoted to the discussion of the use of such mechanical contrivances for preparing bibliographies on chemical subjects. The scientists attending were so absorbed in the possibilities presented that the normal day session was carried on well into the night. In conjunction with some of the large chemical companies the American Chemical Society is continuing to pursue this attractive project.

It seems a pity that such interest and effort cannot be organized to develop and maintain a single standard system whereby many can benefit from the accomplishments of the various individual efforts now isolated in their own narrow activities. The infinitude of detail which can be studied and arranged by particular organizations dealing with a small section of scientific thought might well

benefit all who worked in that field if it were collected in a central agency and coordinated with similar work done by hundreds of similar organizations working in their own particular fields. This system need not be limited to a single type of arrangement for a given subject. There may be alternative classification schedules for a given subject matter to serve different users. With modern equipment these may be solved as simultaneous equations to find, for example, a specific structure used for a particular purpose.

If we carry our dreaming far into the remote future, it is possible to imagine a National Library of Science, patterned after the present Library of the Department of Agriculture, with facilities organized on a scale to embrace all of the fields of scientific and technical effort. With truly modern methods of sorting and filing material, using machines and microphotography, any item of scientific knowledge could be selected almost immediately and furnished to some worker in a position to carry it on to some additional purpose, at a very nominal cost if done on a sufficiently large scale.

The Department of Agriculture Library has already established a very high reputation for service in its field. Any research worker in agricultural subjects is able to obtain very quickly photostatic or microfilm reproductions of practically any published article desired. Specially trained experts are on hand to prepare bibliographies on selected topics for the Department or for other government agencies. Every effort is made to have this type of information readily available.

It is logical to consider such facilities as the Search Room of the Patent Office, for example, as a nucleus of such an ultimate Library of Science. It has a great store of disclosures of inventions which are dedicated to the free use of the public after a few years of exclusive control by the inventor. A system of classification such as is used in the Patent Office, integrated perhaps with the Universal Decimal System, could serve as a basic framework on which to build a really effective system of technical and scientific classification. Such a system would justify a corps of specialists continually supervising its development in accordance with the needs of scientific and technical workers using it. A council of recognized leaders in the major fields of science and technology should meet at periodical intervals to study proposed revisions and guide the efforts of the staff to maintain the system effective and acceptable to all concerned.

Once a system is accepted as standard, the actual labeling of the material with its class and subclass numbers can be done, in many cases, at the source. Magazines and journals, for example, could readily indicate this information with each article, just as the present-day manufacturer of bolts and nuts can indicate on the label pasted on a container the size and number of threads on these useful devices, according to a standard system. Until such complete acceptance is reached, the libraries

and technical societies and organizations can gradually make more and more widespread use of the system in steadily progressive stages.

Such devices as the punched card make it possible to correlate a number of systems of classification, so that a user familiar with any one system may be guided into a mass of information organized according to another system. Over a period of time, the ineffective systems would clearly display their weakness and fall into disuse. Thus, it is unnecessary to contemplate the obviously impossible sudden shift from present systems, representing the accumulated efforts of many years, to a new and different system.

Once we all learn that there are certain effective principles which are most successful in organizing and relating information, and that there are guides and mechanical aids to conduct us from our familiar paths into those chosen by others, much of the bitter controversy as to the respective merits of various systems should disappear. Every creative worker in scientific fields will become a contributor to the development of the eventual standard or truly universal system. When that system is accepted and maintained, time now spent in uncertain hunting for clues to likely sources of information will be substantially eliminated.

Obituary

Leo Černosvitov

1902-1945

Although the common earthworm is one of the most widely investigated of laboratory animals, the number of competent students of oligochaete taxonomy has always been very limited. Perhaps this is due to the intrinsic difficulty of the subject, since the description of each new species is in itself almost an anatomical memoir. Leo Černosvitov, whose untimely death has deprived the scientific world of one of its most promising oligochaetologists, possessed the special qualifications so essential for an investigator in this field of knowledge. He received a thorough training in zoology at the University of Prague, where the subject of his Doctor's dissertation (1927), "La régression physiologique des organes génitaux du *Tubifex tubifex*," prepared him for both biological and taxonomic investigations.

His career was a stormy one, but filled with interest and adventure. Born at Poltava, Russia, in 1902, he fought with the White Army and was later evacuated, as a boy of 17, to Constantinople. In 1921 he went to Prague and received his higher education under the auspices of the Czech Government. After graduation he began an intensive study of the Oligochaeta which culminated in the publication of some 55 papers in this field alone. A survey of the *Zoological Record* shows that he described, in his short and much interrupted career, some 86 new species, exclusive of redescriptions and assignations of new names. His work on oligochaetes was not confined to a limited group or to a restricted geographical region, but, at one time or another, he studied representatives of nearly every family and from many different parts of the world. Among his more important contributions are a long series of articles dealing with the oligochaete fauna

of the Balkan countries, that hotbed of zoological endemism; a systematic revision of the *Enchytraeidae*, as well as numerous shorter papers on this, the most difficult of oligochaete families; and a review of the literature on cavernicolous oligochaetes. He was the first to observe uniparental reproduction in oligochaetes, and his studies on the resorption of spermatozoa are of equally general interest and importance.

In addition to his work on oligochaetes, Černosvitov engaged in many other activities, earning his living in a variety of ways, as lecturer, as entomologist, and as research technician in the Dental Clinic of the University of Prague. As entomologist he visited the Argentine in 1931-32 to study the control of insect pests in the plantations, and as a result of this visit he published several papers on South American oligochaetes. He investigated spruce sawfly for the British Imperial Institute of Entomology and was in Finland, engaged in this work, at the outbreak of the war. Returning to London, he became monitor for the BBC, feeling this to be his most useful service as a refugee alien, on account of his exceptional knowledge of foreign languages.

At the time of his sudden death from a heart attack, on December 15, 1945, he was on the threshold of happiness and security. Four days previously he had been appointed to the staff of the British Museum, and at last it had seemed that he could devote his entire energies to the work that he loved best. It is to be hoped that arrangements can be made for the completion of his unfinished Monograph of the British Oligochaeta, if not for his projected review of the economic importance of earthworms.

GRACE E. PICKFORD

Bingham Oceanographic Laboratory and
Osborn Zoological Laboratory, Yale University

NEWS

and Notes

President Truman on December 31 signed an executive order transferring control of the U. S. atomic energy program from military to civilian hands. The action followed immediately after appointment of Carroll L. Wilson, formerly executive assistant to Vannevar Bush, director of OSRD, as general manager of the Atomic Energy Commission, upon unanimous recommendation to the President by Commission members.

Two other events occurred when the Commission and its General Advisory Committee of Scientists met in Washington during the first week of the new year: 1) J. Robert Oppenheimer, professor of physics, University of California, and wartime director of the Los Alamos, New Mexico, laboratory, was elected chairman of the General Advisory Committee; 2) three labor consultants were appointed by the Commission as part of a program "to insure the soundest possible labor-management relationships."

The labor group included George H. Taylor, professor of industrial relations, Wharton School of Finance and Commerce, University of Pennsylvania, and former chairman of the War Labor Board; Lloyd K. Garrison, New York attorney, former general counsel and chairman of the War Labor Board and until recently dean of the School of Law, University of Wisconsin; and David A. Morse, Assistant Secretary of Labor and former general counsel of the National Labor Relations Board.

The Commission is making plans to assume control of the Manhattan Engineer District, an operation involving about 43,000 employees, and its consultants have already made a preliminary study of industrial relations at the major atomic energy installations.

Mr. Wilson has served as a consultant for the Atomic Energy Commission since its appointment in October. Prior to this and since the end of the war Mr. Wilson was secretary of the State Department Board of Consultants on International Control of Atomic Energy under the chairmanship of David E. Lilienthal,

who is also chairman of the Atomic Energy Commission. Other members of the Board with whom he worked were Chester I. Barnard, president, New Jersey Bell Telephone Company; J. Robert Oppenheimer; Charles Allen Thomas, vice-president, Monsanto Chemical Company; and Harry A. Winne, vice-president, General Electric Company.

Mr. Wilson became executive assistant to Dr. Bush as director of OSRD at the time of its organization after similar work with the National Defense Research Committee and served until the end of the war. He was active in setting up the entire organization, was responsible for coordinating the various branches of OSRD, and directly supervised the Office of Scientific Personnel.

On December 12 President Truman appointed a nine-man General Advisory Committee made up of James Bryant Conant, president of Harvard University and president of AAAS; Lee A. DuBridge, president, California Institute of Technology; Enrico Fermi, professor of physics, University of Chicago; I. I. Rabi, professor of physics, Columbia University; J. R. Oppenheimer, professor of theoretical physics, University of California; Glenn T. Seaborg, professor of chemistry, University of California; C. S. Smith, director, Institute of Metals, University of Chicago; Hartlev Rowe, vice-president, United Fruit Company; and Hood Worthington, chemical engineer, E. I. du Pont de Nemours & Co. (*Science*, December 13 and 20).

The \$1,000 prize awarded by AAAS each year for an outstanding contribution to science presented at the annual winter meeting was divided this year between two research teams.

One-half went to T. M. Sonneborn, Winifred Jacobson, and Ruth V. Dippell, of Indiana University, for their studies on the mechanism of heredity of paramcium.

The other half was awarded to Quentin M. Geiman and Ralph W. McKee, Harvard University, for their method of growing malaria *in vitro*. The latter study began in July 1943, and continued until December 1945, under the direction of Eric G. Ball, of Harvard, and was later continued by the prizewinners under a grant-in-aid from the U. S. Public Health Service. By December 1946, a method was developed for keeping the malaria

parasite alive in test tubes for as long as seven days. Dr. Geiman acknowledged his debt to predecessors on whose work his own was based, particularly that of William Trager, Rockefeller Institute of Medical Research, who in 1941 succeeded in culturing avian malaria.

The Indiana geneticists brought the second recognition to that institution within the past few weeks. H. J. Muller received the Nobel Prize on December 10 for his studies on the influence of X-rays on genes and chromosomes. In 1927, when he was at the University of Texas, this work was awarded the \$1,000 AAAS prize. Dr. Muller, who had flown to Stockholm to receive the prize from King Gustav, returned to the United States on December 22 and attended the meetings in Boston.

In 1936 W. M. Stanley, who shared the Nobel Prize in chemistry this year, won the AAAS award for his biochemical investigation of tobacco mosaic virus proteins.

In 1939, I. I. Rabi, Department of Physics, Columbia University, presented the winning paper on "Radio Frequency Spectra of Atoms and Molecules."

In 1941, the last time at which an annual prize was given at an Association meeting, the award was also divided between three people: Dugald E. S. Brown and Douglas A. Marsland, of New York University, for their paper on "The Reversible Denaturation of Enzymes as a Determining Factor in the Reactions of Biological Systems to Temperature and Pressure" and Frank H. Johnson, of Princeton University, for his closely related paper on "The Mechanism of Temperature and Hydrostatic Pressure Reversal of Narcosis in Luminous Bacteria."

The Association now grants three \$1,000 prizes. Announcement was made in Boston (*Science*, December 27) of a new \$1,000 prize for magazine writers of popular scientific articles; and, as announced previously (*Science*, December 13), the \$1,000 AAAS-George Westinghouse Science Writing Award for newspaper reporting in 1946 was won by James Graham Chesnutt, of the San Francisco *Call-Bulletin*.

About People

Glenn T. Seaborg, professor of chemistry, University of California, and co-discover of plutonium, americium, and curium, was recently selected "Chemist

of the Year" in a national poll of chemists and chemical engineers conducted by the American Chemical Society. Prof. Seaborg, a member of the nine-man advisory committee to assist the United States Atomic Energy Commission, during the war directed research on plutonium and other transuranium elements at the University of Chicago Metallurgical Laboratory (*Science*, October 25).

Charles H. Swift, associate professor and secretary of the Department of Anatomy at the University of Chicago, has retired with emeritus status. Dr. Swift has been associated with the University for the past 47 years.

Robert F. Rinehart has been promoted to professor of mathematics at Case School of Applied Science. Dr. Rinehart has been a member of the Case faculty since 1937. On May 31 he was awarded the Medal for Merit in recognition of his wartime activities with the Operations Research Group of the Navy.

Bascom A. Belcher, formerly a major in the U. S. Army, has been appointed associate agronomist in the Division of Sugar Plant Investigations, U. S. Department of Agriculture, and will be locally in charge of the U. S. Sugar Plant Field Station located at Canal Point, Florida. Mr. Belcher served 11 months as an agricultural economist with Natural Resources Section, GHQ, SCAP, in Tokyo, Japan. At the Canal Point Station, he will carry forward research work associated with sugarcane breeding and sugarcane agronomy.

Joseph E. Greaves, head of the Department of Bacteriology and Biochemistry and on the faculty of the Utah State Agricultural College since 1907, retired from administrative duties on June 30. He continues on the teaching faculty.

C. O. Miller, since 1938 vice-president and scientific director, Lakeside Laboratories, Milwaukee, Wisconsin, has been named president of the Kremers-Urban Company, Milwaukee.

P. H. Waring Webb, formerly at the University of North Carolina, has been appointed associate professor of biology at Coker College, Hartsville, South Carolina.

Edgar Compere, formerly of the Standard Oil Company of New Jersey, Louisiana Division, has been appointed assistant professor in the Department of Chemistry, Louisiana State University.

Edward J. Van Loon was appointed to the staff of the Department of Biochemistry, School of Medicine, University of Louisville, Kentucky, September 1. Since January 1946 he had been an instructor in biochemistry at Michigan State College.

Merrill F. Roff, formerly director of the Aviation Psychology Program, Office of the Air Surgeon, AAF, has joined the staff of the Research Division, Air University School of Aviation Medicine, Randolph Field, Texas, as chief of the Department of Psychology.

Donald P. Costello, professor of zoology, University of North Carolina, has become a member of the Editorial Board of the Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts.

William T. Winne, formerly at Cornell University, has been appointed assistant professor of botany, Union College, Schenectady.

Stephen F. Roach has been appointed instructor in physics at the Jersey City Junior College.

Grants and Awards

Vannevar Bush, president of the Carnegie Institution of Washington, and director OSRD, has been named 1946 winner of the Hoover Medal, awarded jointly by the American Society of Civil Engineers, the American Institute of Mining and Metallurgical Engineers, the American Society of Mechanical Engineers, and the American Institute of Electrical Engineers. Dr. Bush will receive the award January 30 in New York City at the winter meeting of the latter society.

The citation refers to Dr. Bush as an "engineer, educator, and administrator, who, in critical time of need, was in a most special sense an organizer, guiding spirit, and driving force of the nation's achievements in physical and medical science."

The first medal was conferred in 1930

upon Herbert Hoover, for whom the award is named. Dr. Bush is the ninth recipient.

In addition to this award, Dr. Bush holds the Holley Medal of the American Society of Mechanical Engineers and the Edison Medal of the American Institute of Electrical Engineers.

Certificates of Exceptional Service for their "personal contribution" in producing the binocular developed by Bausch & Lomb at the request of the Navy Department have been presented to three employees—J. Donald Dutcher, Henry F. Kurtz, and Willard T. Perkins—who are principally responsible for designing an accurate gunfire control instrument, the submarine telescope, Mark 91.

The American Academy of Arts and Sciences, under terms of a gift of the late Francis Amory, Beverly, Massachusetts, will offer a substantial prize for outstanding work on alleviation or cure of diseases affecting human reproductive organs. The gift provided a fund, the income of which may be awarded at seven-year intervals as a prize and medal to any individual or individuals for work of exceptional merit in this field. If work warranting it has appeared, the next award will be made in 1947 for the most outstanding contribution, as based on published work and recognized accomplishment, in the past seven years. Suggestions aiding the Committee in making a selection may be addressed to Secretary Amory Fund Committee, American Academy of Arts and Sciences, 28 Newbury Street, Boston, Mass.

A Lasker Group Award was presented to the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, "in recognition of distinguished service in the solution of problems involving the health and comfort of the armed forces, with particular reference to insect-borne diseases," by the American Public Health Association, at the 1946 convention of public health workers November 12 in Cleveland, Ohio. F. C. Bishop, assistant chief in charge of research at the Bureau, and over-all director of research conducted for benefit of the armed forces, received the award for the Bureau.

Colleges and Universities

Lehigh University has received a grant of \$60,000 from the War Depart-

ment to make an engineering analysis of bomb damage reports and studies made in World War II.

The United States Naval Academy will hold examinations for positions in the civilian faculty April 4 and 5. Starting salary for science instructors is \$4149.60, for assistant professors \$4400.40, and for associate professors \$4902.00. Application forms and notice of requirements may be obtained from the Superintendent, United States Naval Academy, Annapolis, Maryland. Applications must be submitted before March 15, 1947.

A War Memorial Scholarship of \$1,000 in Mechanical Engineering has been established at Ohio State University by Dr. and Mrs. William Lloyd Evans in memory of their son, William Arthur, a graduate of Ohio State's Department of Mechanical Engineering, lost in action in enemy waters while serving as radar and communications officer aboard the submarine *U.S.S. Tullibee*. Dr. Evans was for many years chairman of the University's Department of Chemistry.

St. Louis University School of Medicine has announced the following recent faculty changes: Percy J. Carroll, Brig. Gen. U. S. Army (ret.), assistant dean and professor of public health; Mark C. Wheelock, University of Alabama, assistant professor of pathology; Paul Murphy, Washington University, assistant professor of clinical medicine; and Benjamin DeBoer, University of Missouri, assistant professor of pharmacology.

The Department of Pharmacology, University of Chicago, is continuing work on the cinchona alkaloids under the direction of E. M. K. Geiling and F. E. Kelsey, under a grant made by Cinchona Products Institute of New York.

The University of Rochester is preparing for a nationwide competition among high school students for five science scholarships valued at \$1,500 each. Fourth annual competition of its type, the contest was inaugurated by the Bausch & Lomb Optical Company, and is open to students in the more than 4,500 high schools and preparatory schools participating in the Bausch & Lomb Honorary Science Awards. Under the latter program, established in 1932 to encourage young people to engage in scientific careers, medals are presented each year to graduating seniors having highest standings in scientific subjects.

Winners of the fourth annual science scholarship contest will be chosen by the University Scholarship Committee next spring from students who received medals during their four years of high school.

Fifteen boys and girls have won \$1,500 scholarships in the past three years. Eleven others stood so high that they were given other university scholarships for nearly equivalent amounts.

Wayne University College of Medicine has been awarded a grant of \$10,000 by the U. S. Public Health Service in support of research on the pharmacology of synaptic function. The project will be carried on in the Department of Pharmacology and Therapeutics under the direction of Amedeo S. Marrazzi, head of the department.

Bowdoin College has recently received three gifts from psychologists: a set of lithoprints of great philosophers and psychologists, donated by Linus W. Kline; and the libraries of the late Charles T. Burnett (formerly professor of psychology at the College) and of the late Edwin B. Holt.

A national survey of the requirement and availability of botanists, prepared at the request of President N. E. Stevens, of the Botanical Society of America, has been completed. The study shows that by 1950, on a conservative estimate, there will be a shortage of 277 botanists with the Ph.D. and 177 with the Master's degree. This is 32 per cent and 46 per cent short of the 1950 anticipated requirements. Copies of the survey, furnished without charge to any who may be interested, may be obtained by writing to: Dr. K. Starr Chester, Department of Botany and Plant Pathology, Oklahoma A. & M. College, Stillwater.

Elections

Charles A. Thomas became president-elect of the American Chemical Society for 1947, according to an announcement from the Council. Dr. Thomas is vice-president and technical director of the Monsanto Chemical Company, St. Louis, and project director of the Clinton Laboratories at Oak Ridge, Tennessee. He was one of the co-authors of the Acheson-Lilienthal report on international control of nuclear power. (See *Science* April 12, 1946.)

The Council also announced the election of four new councilors-at-large for

three-year terms: Cecil L. Brown, director of the Esso Laboratories, Standard Oil Company of Louisiana, Baton Rouge; M. L. Crossley, research director, American Cyanamid Company, Bound Brook, N. J.; Glenn T. Seaborg, University of California, Berkeley; and Roger J. Williams, director, Biochemical Institute, University of Texas.

Roger Adams, a member of the new Publications Committee of AAAS is chairman of the Chemical Society's Board of Directors.

The Royal Society, London, at its anniversary meeting November 30, elected Sir Robert Robinson president, Sir Thomas Merton treasurer, Sir Alfred Egerton and Sir Edward Salisbury secretaries, and E. D. Adrian foreign secretary. Other members of the Council include: C. H. Andrewes, W. T. Astbury, W. Brown, E. C. Bullard, A. C. Chibnall, C. A. Lovatt Evans, N. H. Fairley, R. A. Fisher, S. Goldstein, E. L. Hirst, H. W. Melville, M. H. A. Newman, M. L. E. Oliphant, C. F. A. Pantin, H. H. Read, and Sir Reginald Stradling.

Edward B. Tuohy, associate professor of anesthesiology, Mayo Clinic and Mayo Foundation, was elected president of the American Society of Anesthesiologists, Inc., for 1947 at a meeting in New York City December 12.

Meetings

The 8th International Congress of Genetics will be held in Stockholm in the summer of 1948. Swedish geneticists have formed an Organization Committee for the Congress, with Gunnar Dahlberg, University of Uppsala, chairman, and Gert Bonnier, Institutet för Husdjursförädling, Wiad, Eldtomta, general secretary.

Recent Deaths

Frank Clifford Stockwell, 63, Stevens Institute, died at his home in Hoboken, N. J., December 29. He had been chairman of the Department of Electrical Engineering since 1925.

George Klemperer, 81, retired professor of internal medicine, University of Berlin, where he was active in cancer and metabolism research, died December 24 in Boston. He retired in 1933 and came to the United States in 1935.

Porter James Crawford, 51, director of the Caribbean region of the International Health Division, Rockefeller Foundation, died December 27 in Havana. Dr. Crawford joined the foundation in 1928 and until 1934 studied control of yellow fever in Brazil. During the next three years he worked on malaria control in Panama, and in 1939 was made regional director of the division.

Collins P. Bliss, 80, Scarsdale, N. Y. dean emeritus of the New York University College of Engineering, died December 28 at Tupper Lake, New York.

Christian I. Gunness, 64, head of the Department of Agricultural Engineering, Massachusetts State College, died December 21 of coronary thrombosis.

An Anglo-American agreement for the exchange of German technical information acquired by the two nations since VE-Day has been announced by the Commerce Department. Under the agreement representatives of each nation will be able to select from the other's storehouse of German scientific and technical documents which have been microfilmed. Requests for British documents should be addressed to the Office of Technical Services, Department of Commerce.

The International Committee on Weights and Measures met in Sèvres and Paris October 22-29 for its first session since 1937. Members from Roumania, Sweden and Yugoslavia, who had expected to attend, were unable to do so. From the Japanese member, H. Nagaoka, no news has been received since 1939. The remaining eleven members who took part in the session were Louis de Broglie of France; G. Cassinis, Italy; M. Châtelain, U.S.S.R.; E. C. Crittenden, U.S.A.; M. Dehalu, Belgium; W. J. deHaas, The Netherlands; E. S. Johansen, Denmark; W. Kösters, Germany; Z. Rauszer, Poland; M. Ros, Switzerland; and J. E. Sears, Great Britain. Albert Pérard, director of the International Bureau of Weights and Measures also attended.

Mr. Sears was elected president of the Committee and Mr. Dehalu permanent secretary until the next session in 1948. The position of sous-directeur of the International Bureau was re-established, and Charles Volet was appointed.

The International Bureau was able to continue its work with very little interruption during the war though rising costs and inability of some countries to pay their dues created financial difficulties. To meet these difficulties and also provide for the enlarged scope assigned to the Bureau in recent years the Committee recommended that the General Conference of Weights and Measures scheduled to be held in 1948 arrange for an increase in national contributions to the Bureau.

Technical problems considered by the Committee included practicability of adopting the wave length of some spectral line as the primary standard of length, the definition of units of heat, and revision of the international scale of temperature. Decisions were made to proceed with the adjustments of the units of electricity and light which had been planned for 1940, and introduce the new units into practice as of January 1, 1948.

The electrical units designated as "international" are to be replaced by "absolute" units derived directly from the basic mechanical units and consistent with them within the errors of measurement. The "international" units used in various countries are slightly different, and as a basis for comparisons mean international units were set up at the International Bureau before the war by taking means of the units maintained by the national laboratories of France, Germany, Great Britain, Japan, U.S.S.R. and the United States. The relations accepted by the International Committee for transferring from one system of units to the other are as follows:

- 1 mean international ohm = 1.00049 absolute ohms
- 1 mean international volt = 1.00034 absolute volts

Some questions have arisen regarding maintenance of the mean ohm since 1939, and precise relations of the various national units to the absolute values adopted are not yet certain. Differences from the above factors, however, will generally not exceed 1 unit in the last decimal. For the United States, for example, the conversion factor for volts will be 1.00033, while that for ohms will be approximately 1.00050. This makes the U.S. "international" watt equal to 1.00016 "absolute" or mechanical watts. For electrical measurements this relation is very exact, but the combined uncertainties of measurement of actual absolute magnitudes amount to several parts in

100,000, and the rounded factor 1.0002 may well be used when heat or mechanical energy is to be evaluated. The units of light to be introduced are the "new candle" and "new lumen," the magnitudes of which are fixed by taking the brightness of a black-body radiator at the temperature of solidification of platinum as 60 candles per square centimeter. For light differing in color from that given by this primary standard the values are determined by use of standard spectral luminosity factors previously adopted by the Committee. This new combination makes the primary unit about 1.8 per cent smaller than the old international candle as represented by carbon-filament lamps, but the difference becomes smaller at higher temperatures so that practically no change will be made in the ratings for ordinary tungsten-filament lamps in the United States.—*E. C. Crittenden*, Associate Director, Bureau of Standards, Washington, D. C.

NRC News

The Committee on Research in Endocrinology has announced that requests for grants-in-aid during the period July 1, 1947, to June 30, 1948 will be received until February 28, 1947. In addition to a statement of the problem and research plan or program, applicants are asked to submit information about method of attack, institutional support of the investigation, and uses to be made of the sum requested. No part of any grant may be used by the recipient institution for administrative expenses.

The Committee makes grants-in-aid of research in the general field of experimental and clinical endocrinology. Applications for support of research in the problems of sex in the narrower sense are not administered by this committee and should be directed to the Committee for Research in Problems of Sex of the NRC.

The Committee on Research in Endocrinology will continue to give consideration to the support of studies of the effect of sex hormones on nonsexual functions, e.g., on general metabolism and metabolism of steroid hormones. Application blanks may be obtained by addressing the Secretary, Division of Medical Sciences, National Research Council, 2101 Constitution Ave., Washington 25, D. C.

TECHNICAL PAPERS

Hypervolemia in Mice Bearing Transplantable Granulosa Cell Tumors¹

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It has recently been reported (1) that the livers of mice bearing transplantable granulosa cell tumors of the ovary are extremely congested and are greatly increased in weight. Microscopically, it is seen that the liver sinusoids are extremely distended with blood, so that their width is several times that of the cords of the liver cells. At the advanced stage of this condition the liver cells undergo atrophy; some vessels become thrombosed, and necrosis of liver tissue supervenes. The hyperemia is not restricted to the liver, for the adrenal, ovary, and spleen are sometimes also extremely congested; but no organ above the diaphragm has been noted to be involved.

The massive hyperemia suggested an increase in the blood volume. This was determined by adapting the exsanguination-perfusion technic and the Evans blue (T-1824) technic to the mouse.

The data, summarized in Table 1, indicate a huge increase in

TABLE 1
BLOOD VOLUME IN MICE

Mice	Exsanguination-perfusion technic			Dye technic		
	No. in group	Body weight %		No. in group	Body weight %	
		Extremes	Average		Extremes	Average
Normal	12	3.5-6.6	5.2	9	9.0-12.7	10.9
With granulosa cell tumors	24	7.3-35.8	13.6	10	9.7-54.6	34.3
With other tumors	10	3.7-11.0	6.9	6	5.9-12.4	9.0

the blood volume of animals bearing granulosa cell tumors, heretofore unknown to occur in any condition. Mice bearing any of four different types of tumors of comparable mass and vascularization possess blood volumes that do not differ appreciably from those of normal mice. This huge increase in blood volume is apparent even after simple exsanguination.

The hematocrit values are normal, slightly increased, or decreased indicating that there is an enormous increase in both plasma and red cells.

There is a direct relationship between blood-volume values and congestive changes in liver as seen in sections. In mice with marked congestion, the blood-volume values, as determined by the exsanguination-perfusion technic, were 3.7-17.2 cc. (12.3-35.8 per cent of body weight); with moderate congestion, 2.0-9.4 cc. (7.3-21.3 per cent of body weight); and

with slight congestion, 2.0-3.8 cc. (7.6-10.8 per cent of body weight), as compared with 1.3-2.1 cc. in normal mice and 1.2-3.8 cc. in mice bearing other tumors.

It is possible that these granulosa cell tumors secrete a substance, as yet unknown, or a substance already known, which, when produced in ever-increasing amounts as the tumor grows, causes the hypervolemia. Such a substance is being sought.

It is possible that the vasodilatation accompanying hypervolemia is caused by an excessive amount of the vasodilator material described in *Science* by Shorr, Zweifach, and Furchgott (1945, 102, 489) and that the phenomenon is a disturbance of the homeostatic vascular mechanism related to shock.

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Streptomycin as an Aid in Isolating Influenza Virus¹

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Hirst (3) used penicillin to thwart bacterial contamination in the isolation of influenza virus from unfiltered throat washings inoculated into the amniotic sac of the developing chick embryo. This same method was used by us to advantage in the type B influenza epidemic of November 1945.

Nose and throat washings obtained from three patients on November 20 were pooled, frozen on solid CO₂, thawed, ground in a mortar, and centrifuged. To each milliliter of unfiltered washings 500 units of penicillin were added, and 0.1 ml. of the mixture was inoculated into each of 24 twelve-day-old embryonated eggs. Of the 24 inoculated, 8 were alive after two days incubation. The live, embryonated eggs were tapped after two days incubation at 35° C. and again on the third day, and were found to contain influenza virus, type B, by Salk's (4) modification of the Hirst (2) hemagglutination and anti-hemagglutination test.

Because of the high death rate of the embryos experienced above, we added 1,000 units of streptomycin/ml. of washings in addition to the 500 units of penicillin. By use of both antibiotics we reduced our losses to less than 10 per cent of the inoculated embryos.

Subsequently both type A (isolated in Iowa City from 1943 epidemic) and type B (current strain) were tested in embryonated eggs to determine whether the streptomycin-penicillin combination interfered in any way with their propagation. We found as much virus produced in the presence of the antibiotics as with control eggs containing no streptomycin or penicillin.

Since that time we have used the streptomycin-penicillin

¹ This investigation was aided in part by the Commission on Influenza Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Washington, D. C.

This investigation was supported by the Donner Foundation, the Anna Muller Fund, the National Advisory Cancer Council, and the Jane Coffin Childs Memorial Fund for Medical Research.

combination on a number of unfiltered specimens, including nose and throat washings, sputum specimens, and 10 per cent lung suspensions, when amniotic sac inoculations were desired. The death rate of the embryos and the incidence of positive cultures from embryonic fluids have been considerably lower when the two antibiotics were used than when penicillin alone was used. In fact, the occurrence of bacterial contamination when both antibiotics were used has been uncommon. One example has been encountered in which the combined antibiotics failed to protect the embryos in any degree. In this instance we inoculated embryonated eggs with a 10-per cent lung suspension from a human case of suspected influenzal pneumonia terminating fatally. All embryos died in 24 hours. The bacterial contaminant was a strain of *Pseudomonas pyocyanea*, which proved to be very resistant to streptomycin.

In some of our virus studies we have had occasion to pass mouse lungs at short intervals (24-48 hours) for a number of passages. Almost without exception we have encountered difficulty from bacterial pneumonias. However, when passages were made at 96-hour intervals, this difficulty was not encountered. We have assumed that these pneumonias arise as a result of washing bacteria from the upper respiratory tract of the mouse into his lungs as a result of the intranasal inoculation. The 24- and 48-hour intervals do not allow sufficient time for the mice to eradicate these organisms, and the rapid passages probably increase the virulence of these bacteria to the point at which they can kill the animal. Almost without exception the offending organism has been an alpha-hemolytic streptococcus or a small gram-negative rod (not *Salmonella*). Recently we have obviated the difficulty encountered in rapid lung passages by the addition of 500 units of penicillin and 1,000 units of streptomycin/ml. of 10 per cent mouse lung used for passage. The antibiotics and lung suspension are usually allowed to contact each other for about one hour before inoculations are made.

TABLE 1
CULTURES OF MOUSE LUNG

Time of sacrifice after intranasal inoculation	With antibiotics		Without antibiotics	
	Aerobic	Anaerobic	Aerobic	Anaerobic
24 hours	No growth	No growth	50 colonies*	32 colonies
48 hours	4 colonies	No growth	144 colonies	400 colonies

* The colonies consisted of nonhemolytic gram-negative rods, alpha-hemolytic streptococci, and anaerobic streptococci.

To check on the above technic we have inoculated two groups of mice (10 in a group) with 10 per cent normal mouse lung suspension intranasally. In one group the two antibiotics were added to the normal lung suspension before the inoculation. The other group served as a control. After 24 hours five mice from each group were sacrificed, their lungs pooled and ground to a 20-per cent suspension in saline, and one loopful of the suspension cultured aerobically and anaerobically. The remaining mice were tested in 48 hours. The results are shown in Table 1.

Addendum. Since submission of this article to the Surgeon General's Office for publication an article has appeared (1) in which the authors reported that they found streptomycin

without toxicity for chick embryos and that there were no untoward effects on influenza virus, types A and B.

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Meteorite Impact Suggested by the Orientation of Shatter-Cones at the Kentland, Indiana, Disturbance

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A large quarry about two miles east of Kentland, Newton County, Indiana, exposes the center of a well-known local area of intensely deranged Paleozoic beds in a region of essentially flat-lying strata. It is generally accepted that a violent natural explosion in the geological past formed this (3, 4) and similar disturbances (2, 5). The application of violent shock in the formation of the Kentland disturbance is indicated in part by the jumbling of the strata, the shattering of the limestone, and the pulverization of the sand grains of the St. Peter sandstone. Although there is no indication of the presence of igneous material or of hydrothermal alteration, Bucher (3) and Shrock (4) have ascribed the origin of this disturbance to a deep-seated explosion of gases derived from an igneous intrusion. The observation made recently by the present writer may invalidate such a cryptovolcanic explosion hypothesis or any other suggested mode of origin that involves a deep-seated force acting from below the strata.

Prominently developed in the limestones at the Kentland disturbance are cup-and-cone structures called "shatter cones," "pressure-cones," or "shear-cones," which were apparently produced by the explosive shock which formed the disturbance. These curious shatter-cones are present in two other explosion disturbances of the Kentland type but are not reported from other types of geological disturbances. If well developed, these structures consist of a large primary cup-and-cone which has a surface grooved in such a manner as to appear as a series of smaller secondary, overlapped and imbricated half-cones, with the apexes of these secondary features pointing toward the apex of the primary cup-and-cone. This convergingly-grooved surface is a fault surface similar to the parallel-grooved slickensided surfaces common in fault zones. An examination of the cup-and-cone surface and of the base of some of these features reveals that the cup is displaced relatively downward, i.e. away from the apex, with respect to the cone, so that these are normal faultlets rather than thrust faultlets. By applying Hartman's law, which states in part that under nonrotational forces the acute angle formed by shear planes in brittle material is bisected by the axis of maximum stress, it is apparent that the axis of such a primary cup-and-cone is also the axis of maximum stress. An examination of many of these structures at Kentland revealed that the axes of the primary cup-and-cones are invariably oriented normal to the bedding and the apexes point toward the base of the bed. The common orientation of the apexes indicates

that the deforming stress was unidirectional. A consideration of the mechanics of this type of deformation shows that the cup was active and the cone passive, and that the shock was applied from the direction in which the apexes point. Therefore, the orientation of the shatter-cones suggests that, assuming that the beds were essentially horizontal prior to deformation, the shock force resulted from some type of explosion directly above the beds rather than from a cryptovolcanic explosion below the beds.

A probable interpretation for the observed orientation of these shatter-cones is that the Kentland disturbance is the "root" structure of a meteorite crater which was formed after late-Paleozoic time and deeply eroded prior to the Pleistocene. In fact, it is difficult to conceive of any other type of explosion than that of a large meteorite which would act from above the strata. Boon and Albritton (1) have developed evidence to show that structures of the Kentland type are the product of a meteorite impact. According to these writers, high-velocity impact, many times faster than the velocity of a shock wave in any type of rock, compresses the rocks elastically, rather than deforming them plastically, after which they are "back-fired" into a damped-wave disturbance. Shatter-cones pointing toward the impinging body may be formed during the initial or compressional stage of such a meteoroid impact.

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Evidence That the Hemolytic Anemia Caused by Fat and Choline Is Not Due to Lipotropic Action¹

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Johnson and co-workers (6, 7) have shown that the feeding of fat to dogs increases the fragility of their red blood cells and causes an increased destruction of erythrocytes as judged by an increased output of bile pigment. The hemolytic agents from fat are presumably fatty acids and soaps which have escaped resynthesis into neutral fat during absorption (4, 5). Although a high fat diet does not by itself produce anemia, Dupee, *et al.* (3) have shown that it can produce hyperplasia of the red bone marrow in dogs.

We have demonstrated (1, 2) that the administration to dogs of choline chloride in addition to a high fat diet causes the rapid development of an acute hemolytic anemia, accompanied by a rise in the icterus index, which rapidly regresses upon cessation of administration of either the fat or the choline (if the latter has not been fed for more than 8 or 10 days). To explain the mechanism of the production of this anemia, we have postulated that the choline exerts a "holding" action upon the bone marrow to prevent any great increase in its rate of erythropoiesis, while the fat furnishes products which cause the actual hemolytic destruction of erythrocytes.

¹ Research paper No. 826, Journal Series, University of Arkansas.

Our experiments did not preclude the possibility that choline might exert its lipotropic action with the fat and thereby, in some manner, produce anemia. The present investigation was made to test this possibility, by the administration of atropine, which does not antagonize the lipotropic action of choline, but does block its pharmacologic vasodilator action.

Normal erythrocyte counts and hemoglobin percentages (Sahli) were determined, over a period of days, on one splenectomized and two normal dogs. The three animals were then given 60 grams of fat,² and 10 mg./kg. of choline chloride daily, in addition to their regular adequate diet. After the first day of the experiment, two of the dogs were given daily subcutaneous injections of atropine sulfate (0.5 mg./kg.) in addition to the orally administered choline and fat. Erythrocyte numbers and hemoglobin concentrations were determined daily on the blood of each dog during the experimental period.

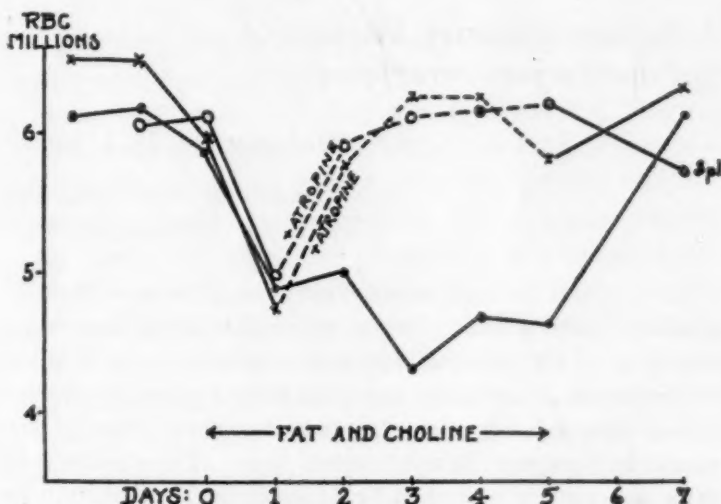


FIG. 1. The effect of atropine upon the erythrocyte counts of dogs rendered anemic by fat and choline. Dashed lines indicate periods during which atropine, in addition to fat and choline, was administered to two dogs. Open dot line (labeled Spl.) represents erythrocyte numbers of a dog which had been splenectomized about one year prior to this experiment.

It will be seen in Fig. 1 that the feeding of fat and choline caused reductions in the erythrocyte numbers of the three dogs, as observed about 24 hours after the first daily doses. At this time, two of the dogs were started on daily doses of atropine in addition to the choline and fat, and as a result, their erythrocyte numbers (dashed lines, Fig. 1) returned to normal within two days. The third dog, whose erythrocyte count is recorded as a solid line throughout Fig. 1, did not receive atropine and remained anemic during the period of fat and choline feeding. Hemoglobin percentages were observed to change proportionately to the erythrocyte counts.

Since atropine was shown, previously, to prevent or abolish the hyperchromic anemia which was produced more slowly by the administration of choline alone (1), we rather expected that it would also antagonize the hemolytic anemia induced by fat and choline, as it actually did in this experiment.

We believe, therefore, that in these experiments, choline acts by the mechanism previously postulated (1), *i.e.* as a weak brake to inhibit any acceleration of erythropoiesis which may normally follow the hemolytic destruction of red blood cells. It probably does this by causing vasodilation and improved

² Wilson's "Advance," a shortening made from animal and vegetable fats.

blood and oxygen supply to the bone marrow, thus tending to depress erythropoiesis.

It is concluded that choline aids in the production of this anemia, *not* by virtue of a lipotropic action, but rather by its vasodilator or pharmacological action.

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A Nonrespiratory Variant of *Saccharomyces cerevisiae*¹

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In studies of the toxic effect of ethylene oxide on a strain of *Saccharomyces cerevisiae*, it was noted that, at a time when almost all of the yeast cells in a suspension have been killed by the poison, some of the surviving cells give rise to colonies unlike those of the original organism. These colonies are smaller and rougher than the normal type. The organisms in them are usually more spherical, somewhat smaller, and in some cases occur in clusters. When restreaked on agar plates, they gave rise to colonies showing much variation in size. The ethylene oxide-induced variants can be obtained repeatedly from the original strain after repurification of the latter and thus do not represent a constant degree of contamination.

The occurrence of mutated colonies has been described before (2, 3) and interpreted as being due to the development of haploid yeast cells. However, such variation is usually observed only after sporulation of the yeast is followed by isolation on special media or by dissection and cultivation of individual ascospores.

In a few instances we have observed what appeared to be fusion between cells of the variant colonies to form a diploid yeast. Haploid yeasts are often unstable, not only because of possible conjugation but also because of the high rate of mutation to which they are subject. Instability has been noted in the variants obtained in this work and has led to difficulties in studying them. However, certain strains of the apparently haploid type have been sufficiently stable to be examined in some detail, and one, which did not undergo further variation over a period of several months, has shown very interesting characteristics.

Unlike suspensions of the parent strain, resting suspensions of this variant show no ability to oxidize either glucose or alcohol under aerobic conditions. In any case, the respiration

is so slight that it is not measurable in Warburg respirometers. This is true even when the organisms have been grown in a shaking liquid medium containing 0.5 per cent glucose and 5 per cent yeast autolysate and have been harvested during the period of exponential growth. As will be seen in Table 1, the rate of fermentation is high, and no Pasteur effect can be observed.

Suspensions of the parent yeast examined spectroscopically show absorption bands corresponding to reduced cytochromes A, B, and C at 605, 552, and 563 A. Similar suspensions of the variant show a strong band at 563 A. (cytochrome C), but lack the other two even when reducing agent is added in excess.

TABLE 1

RATES OF RESPIRATION AND FERMENTATION OF PARENT AND VARIANT STRAINS AT 30° C. WITH 2 PER CENT GLUCOSE AND 2 PER CENT KH₂PO₄

	Parent	Variant
Q _{O₂}	42.5	0
Q _{CO₂} ^{air}	62	274
Q _{CO₂} ^{CO₂}	145	268

* Only aerobic fermentative CO₂ production included in Q_{CO₂}^{air}.

In addition, the variant gives no test for indophenol oxidase with Nadi reagents according to the method of Keilin (1). The parent strain gives a strong positive reaction under identical conditions. Spectroscopic examination for cytochrome oxidase was not made.

The variant can ferment the same sugars as the parent strain and use them as substrates for growth. As might be expected, however, it does not grow with alcohol as carbon source, although the parent strain is able to do so. Morphologically, it differs in size and in the shape of the cells (parent—(4.2–7.2) × (5.5–10.8) μ; average: 5.5 × 7.2 μ; variant—(4.2–6.6) × (4.8–8.4) μ; average: 5.3 × 6.2 μ). While the thallus of the parent strain occurs as single cells and pairs, that of the variant consists of clusters of from 3 to 20 cells. In old liquid wort cultures, the variant shows no surface growth or ring formation. There are also noticeable differences in gross morphology of streak and giant colonies. The variant has lost the ability to form ascospores.

The question as to why these haploid variants, if such they are, appear after ethylene oxide treatment has not been satisfactorily answered. It might be the result of a specific metabolic effect of the poison on the yeast cells, but it seems more likely that haploid cells or spores may be present in the normal cultures and survive the treatment with ethylene oxide better than the diploid cells because of their slightly greater resistance to the poison. Since the particular variant discussed occurs in clusters of various sizes, it is difficult to compare its death rate in ethylene oxide with that of the original strain by the use of viable counts. Since any difference in resistance cannot be great (all of the yeast cells are eventually killed by ethylene oxide), they are difficult to establish by ordinary statistical methods. However, in studies with another variant, which had similar physiological characteristics (*i.e.* absence of oxida-

¹ A report on a joint research project of the Quartermaster General's Office, U. S. Army, and the University of California. The authors wish to express their appreciation to Prof. Gordon Mackinney for his aid with the spectroscopic work and to Miss Ruth Alleman for technical assistance.

tive metabolism) but which did not form clusters, an apparently slightly greater resistance to the poison was found than with the parent strain.

Castor (4) has described a nonrespiratory yeast variant resulting from treatment with HCN. In contrast to our variant, his organisms lacked cytochrome C. Ethylene oxide does not appear to act as a specific respiratory poison. However, although its effect on the metabolism of yeast, unlike the effect of HCN, appears to be general, it does inhibit fermentation more rapidly in the presence of air than in its absence. Not all of the morphological variants appearing after ethylene oxide treatment lack oxidative metabolism. Several of these showed QO_2 values comparable to that of the original strain.

If greater resistance to the poison explains the appearance of such variants, it may be that only certain types of haploids will be found by this method. To study all of the types found, particularly in view of their further mutation, would be out of the scope of our work. However, the strain discussed, providing its physiological characters remain stable, offers many interesting possibilities for investigation.

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The Deposition of C^{14} in Bone¹

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In the course of experiments on the mineral metabolism of bone salt in rats, we made some observations on the deposition of C^{14} in bone which are worth recording now, although our studies are incomplete.

Beginning in July 1945, we injected a number of rats intraperitoneally with $BaCO_3$ or $NaHCO_3$ in which 75-150 μ c. of C^{14} were present. Some of the rats (weighing 52-75 grams) had been on a normal diet; others, on a low-phosphorus, vitamin-free diet before the injection. All animals were on a normal diet thereafter. The rats were killed at 3 days and 2, 4, 8, and 16 weeks after injection. Among the controls were rats injected with P^{32} or Sr^{89} . The bones, after fixation in absolute alcohol and embedding in nitrocellulose, were sectioned *without decalcification* by the routine of McLean and Bloom (1). Autoradiographs of these sections as well as of the alcohol-fixed soft tissues were prepared for us by Mr. George Svihla. We

found no differences in the results dependent on the normal versus the low P diets.

As was expected from previous studies on the deposition of bone salt as shown by staining with $AgNO_3$ and by the use of Sr^{89} and other calcium substituting isotopes, we found in the autoradiographs of the long bones of rats killed after 3 days that Sr^{89} deposits most heavily in the metaphysis (extending through the calcified cartilage and all of the spongiosa) and is present in smaller amounts in the bone structures of diaphysis and epiphysis. These differences in the amount of Sr^{89} in the several parts of the bone can be accounted for by the occurrence of two processes: (1) a fresh deposit of bone salt in the zone of new growth of bone in which Sr^{89} substitutes for some of the Ca atoms, and (2) an interchange between Sr^{89} and Ca of the bone salt previously deposited in the bone.

Autoradiographs made from rats injected with P^{32} show that P^{32} is deposited in much the same situations in bone as Sr^{89} .

The autoradiographs of the bones of rats injected with C^{14} show a markedly different picture from those of the Sr^{89} rats. Those from rats killed 3 days after injection of C^{14} show the shaft of the bones as black lines. The bone in the epiphysis is a faint gray, while the metaphysis of the growing end of the bone is negative, or practically so, leaving a gap of about 2 mm. between epiphysis and diaphysis. The nongrowing end of the bone is completely outlined in gray. The marrow cavity is so pale that it is probably negative. The autoradiographs of the 2-, 4-, 8-, and 16-week specimens show essentially the same picture as those after 3 days, except that the bones have grown in length and width. With the growth in length, the unblackened zone at the metaphysis increased to about 3 mm. at 2 weeks, 4 mm. at 4 weeks, 10 mm. at 8 weeks, and 12-17 mm. at 16 weeks. Since the blackened lines representing the lateral extent of the diaphysis at the time of injection are still present after 16 weeks, it would seem that the marrow cavity did not increase much in diameter at the site of deposition of radioactive carbon. However, new bone was deposited externally so that the bone as a whole increased in thickness.

Sections of the liver and kidney gave fairly intense autoradiographs at the 3-day and 2-week stages, but were negative after the longer intervals. Since the films were exposed for the same length of time, it would appear that there was approximately as much C^{14} in the bones after 16 weeks as after 3 days, while there was a great decrease in the C^{14} content of the soft tissues. Organ analyses are now being made.

From the autoradiographs of bones of these few rats we would conclude that C^{14} injected as carbonate appears primarily in those areas occupied by pre-existing bone. It does not appear in appreciable quantities in the areas of most recently deposited bone salt. This observation demands further study on the carbon metabolism of bone.

Since the C^{14} content of the bones did not decrease appreciably in 4 months, we believe that the health hazards involved in working with this isotope must be studied, particular attention being paid to the possible development of bone tumors.

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¹ This work was carried out under contract between the University of Chicago and the Manhattan District, Corps of Engineers, War Department.

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IN THE LABORATORY

Apparatus for Studying Crystal Formation

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In an attempt to develop methods for studying some of the problems of mineral deposition, we have designed a simple apparatus which promises to be very useful in many different kinds of studies of crystal growth. Most recorded laboratory experiments on crystallization have made no attempt to simulate the conditions under which many crystals are formed in fissures and cavities in the earth. One of the most common mechanisms under natural conditions is precipitation by slow cooling from moving solutions. The apparatus is a first attempt to design an approximate earth model in which the effect of variables can be studied accurately.

The essential features of the apparatus are shown in Fig. 1. The material to be precipitated is ground, sized, dried, and weighed, then put in the pervious basket in the left arm above

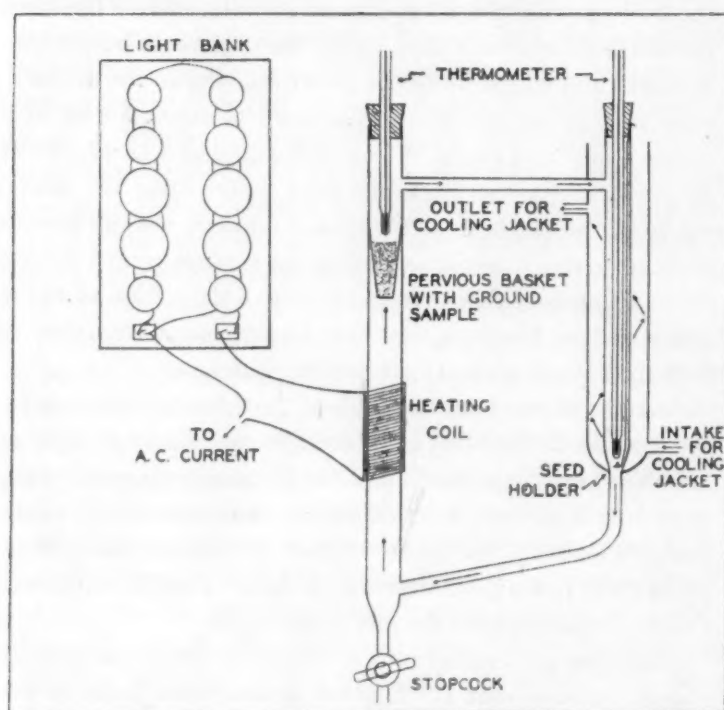


FIG. 1

the heating coil. The heating coil is connected, and convectional circulation established as shown. The temperatures of the two arms can be controlled by adjusting the amount of heat by using various bulb combinations in the lamp bank and by adjusting the rate of flow of cooling water in the jacket surrounding the right arm. After a constant temperature gradient has been established and the solution is saturated at the lower temperature, a seed crystal is introduced at the coldest point of the right arm, and growth on the seed begins.

Many different kinds of studies can be made with the unit. Some of the obvious advantages over ordinary static precipitations might be mentioned. Operation is continuous, and the

system is so stable that little adjustment is necessary after an original temperature setting. The apparatus is especially suited for studies of slightly soluble materials, since thousands of liters of solution can be passed through the sample. Preliminary work indicates also that many slightly soluble substances, which precipitate colloiddally when formed by the mixing of ionic solutions, will crystallize fairly readily down a gentle temperature gradient such as that provided in the apparatus.

The device is now being used to study the effect of flow on crystal orientation and growth, the use of inclusions in crystals as a criterion of temperature of formation, and the rates of leaching of very slightly soluble minerals as a function of temperature. Many other possibilities suggest themselves, such as the response of crystal habit to different temperature gradients and the nature of replacement processes. A second model has been designed with adaptations to fit our specific purposes. This new model will have a plane-sided chamber at the point of crystal formation, so that the crystals can be photographed during growth and the prints pieced into a motion-picture film. In the first design, rate of flow and temperature differential of the two arms are interdependent, and the rate of flow changes with the grain size and total amount of material introduced for leaching. In the new design, rate of flow will be controlled by a diaphragm with adjustable permeability, so that flow and temperature differential can be kept constant, even though the permeability of the sample changes during the leaching process.

Antigenic Carbohydrate-Lipid Isolated From Paraffin-Oil Extract of Dead Tubercle Bacilli¹

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A carbohydrate-lipid complex isolated from tubercle bacilli has been found to induce antibodies when injected into normal animals.

The experiments have been carried out, using as tools the two fractions of biological significance which were obtained from a paraffin-oil extract of dead tubercle bacilli (1). One fraction is called the "toxic" fraction, since as small an amount as 2 γ is sufficient to produce lesions in the lung of normal guinea pigs when injected intraperitoneally in paraffin oil. The other is called the "sensitizing" fraction, as it gives to normal animals definite hypersensitivity to old tuberculin (and also to the whole bacterial cells) when injected intraperitoneally, in oil, in amounts as small as 0.1 mg. Both fractions were found in the oil extract. This oil extract was made in an attempt to elucidate the mechanism by which paraffin oil enhances the ability of dead bacilli to produce

¹ This study was carried on under a grant from the Josiah Macy, Jr. Foundation.

hypersensitivity and lesions. We presumed, and we found, that the oil extracts these fractions from the bacilli. Both were precipitated from the oil with dioxane.

The "toxic" fraction is the chloroform-soluble portion of the precipitate after it has been thoroughly washed with dioxane and methanol. It is a polysaccharide ester of mycolic acid, which still contains, after purification, 1.05 per cent of nitrogen and 0.4 per cent of phosphorus.

The "sensitizing" fraction is that part of the precipitate which is insoluble in the usual organic solvents, such as methanol, ethanol, ether, chloroform, benzene, and petroleum ether. It contains a large amount of protein. (We found later, when we tried to purify these fractions, that each one of them is not free of the other.)

Preliminary experiments led us to believe that the "sensitizing" material acted also as a protective antigen. A clear-cut acquired resistance was obtained in normal guinea pigs which had been previously immunized by the "sensitizing" fraction and which were then injected with 0.1 mg. of living bacilli of low virulence, H-37.

In further experiments the normal guinea pigs were immunized with a more purified "sensitizing" material. The hypersensitized animals were then given bacilli of higher virulence (H-160 Corper). They showed a certain degree of acquired resistance, not only in survival times but also by the degree of tuberculosis involvement of their organs. But in spite of the fact that in four successive experiments, each one involving at least 15 sensitized animals and 15 controls, we lowered the infecting dose to 1/50,000 mg., these animals failed to show the same degree of acquired resistance as that shown by the animals which were sensitized by the less purified material.

It was clear that something else in the "sensitizing" fraction than the sensitizing antigen might be responsible for the previously observed acquired resistance.

On the other hand, animals which were injected with the "toxic" material alone in oil showed an excellent acquired resistance against infection when they received living bacilli more than three months after they were injected. The effective dose of "toxic" fraction was 1 mg. in one single injection, or 2 γ in two successive injections at an interval of a few weeks.

Moreover, there was some evidence that the "toxic" material, the carbohydrate-lipid component, produced antibodies. Guinea pigs immunized with this "toxic" material, as well as animals infected with tubercle bacilli, reacted more to the intracutaneous injection of a few gamma of the "toxic" material than did normal controls.

These observations led us to believe that the acquired resistance developed in guinea pigs by the "sensitizing" material was due to contamination of this material with the carbohydrate-lipid complex.

Our speculations received considerable support when we observed that the water-soluble portion of the hydrolysate² of our carbohydrate-lipid complex reacted strongly, in precipitin tests, with the sera of rabbits and guinea pigs which had been injected with the "toxic" carbohydrate-lipid complex alone. Precipitations were obtained in dilutions as high as 1:10,000,000 with some sera. Strong precipitations were also obtained with the sera of a horse which had been immunized with whole tubercle bacilli and also with the sera of rabbits

that had been immunized with human and avian tubercle bacilli, grown in the "tween 80" medium recently described by R. H. Dubos and B. D. Davis (2).

This is the first demonstration that a chloroform-soluble carbohydrate-lipid complex isolated from tubercle bacilli is antigenic, when injected into normal animals in paraffin oil. Our experiments showed that this carbohydrate-lipid complex aids the process of acquired resistance to the tubercle bacillus. Whether or not this complex is the essential immunizing antigen of the tubercle bacillus remains to be seen.

In any case, it will be a useful tool—a test—which may allow us to follow, *in vivo* as well as *in vitro*, the carbohydrate-antibody formation in the course of infection with the tubercle bacillus.

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A Technique for Obtaining Quickly Permanent Mounts of Nonembedded Botanical Material

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Plant pathologists and botanists are often embarrassed by being unable to preserve permanently material that has not undergone the classical technique of paraffin embedding. The former, for instance, of which the writer is one, would often wish to keep permanent mounts of such material as leaf scrapings or small fragments of bark, fruit, or freehand sections. These materials are usually preserved in a 7 per cent aqueous solution of potassium hydroxide. The mounts can be kept only temporarily even with asphalt lac seals.

As everyone knows, nothing can compete with balsam for permanent mounts, but with such material as is described above it is impossible to follow the classical procedure and, if this is not the case, one cannot always devote the time necessary for such inclusions.

The writer has adopted the following technique to circumvent the difficulty with most satisfactory results:

Slides that are worth keeping are first treated with chloralphenol (see M. Langeron. *Précis de microscopie*. (5th ed.) P. 607). This medium has the property of being miscible to water and balsam. Moreover, it is an excellent clarifying medium, and its miscibility to water makes it an excellent dehydrator. Droplets of this liquid are deposited close to the cover slip while the aqueous medium is pumped out with strips of filter paper. The slides are then slightly warmed to hasten the departure of air. The writer has found it convenient to lay them on the grating of a microscope lamp resistance. Levigation with chloralphenol is repeated until one can have the assurance that all the water is gone. Droplets of Canada balsam solubilized in xylol are then dropped on the site where chloralphenol has been previously deposited, the latter being in turn pumped out from under the cover slip with strips of filter paper. The slides are then heated at a slightly higher temperature to evaporate the chloralphenol. This must be

² Hydrolysis made in methanol 10 per cent potassium hydroxide.

followed closely so as to add fresh droplets as the xylol and chloralphenol evaporate. Finally the material is mounted in pure balsam and can be kept indefinitely.

Counterindication of this technique holds only for hyaline spores or mycelia which are scarcely visible in balsam. But wherever a slight coloration or a sufficiently thick membrane is present, details are sufficiently visible to permit taxonomical or anatomical observations.

There is another drawback to this technique when free spores are present on the slide. These can be displaced or drawn out from under the cover slip when the slide is levigated with the two media. When such is foreseen, one can place the material directly in a mixture of chloralphenol and balsam diluted in xylol in the proportion of two droplets to one. The chloralphenol insures the clarification of the material and the penetration of the balsam. With the cover slip on, the slide is gently heated until the chloralphenol has completely evaporated. Here again, attention must be paid to feeding fresh droplets of Canada balsam as the mounting medium dries up.

This latter procedure does not apply to fresh material but only to herbarium material or one that has been dried beforehand.

This technique has been very useful to the writer to prepare quickly permanent mounts of abundant herbarium material that had to be examined at short notice, while keeping a permanent record of the observations.

Chloralphenol is prepared by mixing by weight two parts of chloral hydrate to one part of phenol crystals. The mixture, liquefied by gently heating it on a flame, can be kept in dropping bottles.

This technique may not be totally unknown, as Langeron seems to infer. In any case, the writer has seen no reference in the botanical or phytopathological literature to its having been used.

Fluorophotometric Determination of Rutin and Other Flavones

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A great deal of interest has developed in the therapeutic use of rutin for the treatment of reduced capillary resistance in certain hemorrhagic diseases (1, 3). Rutin is a flavone glucoside which yields quercetin, glucose, and rhamnose on hydrolysis with dilute acids (2). Most flavones react with boric acid to form highly colored derivatives which can be adapted to colorimetric determinations (5, 6). We have applied the boric acid reaction to the determination of rutin and have also improved the sensitivity of the method by measuring the intensity of fluorescence with a photometer, instead of measuring the optical density of the colored solution. We have also found that the fluorometric method can be used to advantage with purified quercetin, and presumably with other flavones which react with boric acid.

A stock solution of recrystallized rutin¹ in dry acetone was

¹ The rutin used in these experiments was obtained through the courtesy of Dr. M. J. Copley, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia.

diluted to give a series of standards containing known concentrations of rutin. The boric acid reagent was prepared by mixing equal volumes of a saturated solution of boric acid in acetone and a 10 per cent citric acid solution in acetone, as described by Wilson, Weatherby, and Bock (6). The reagent was added to the rutin standards in the ratio of four volumes to one of rutin solution. Colorimetric measurements were made with a Klett-Summerson photoelectric colorimeter using a blue filter (No. 42), and the fluorescence measurements were made with a Pfaltz and Bauer Model B fluorophotometer. The mercury vapor light in the fluorophotometer was passed through a set of filters having a peak transmission in the violet band at 430-440 mμ. The degree of fluorescence was measured with a photocell through a yellow filter having a sharp cutoff at 520 mμ. Fluorescence was also observed using ultraviolet light below 370 mμ; but the photometer readings were higher with the violet light source which was finally chosen for this work. The rutin-boric acid solutions exhibited a green fluorescence which, on examination with a spectroscope, appeared to extend from 510 mμ to 620 mμ.

Calibration of the fluorophotometer with rutin standards produced the results shown in Table 1. The degree of fluorescence appears to be directly related to the concentration of

TABLE 1
COMPARISON OF FLUORIMETRIC AND COLORIMETRIC READINGS WITH
RUTIN-BORIC ACID SOLUTIONS

Rutin/ml. sample (μg.)	Colorimeter readings	Fluorimeter readings
10.0	27.0	67.0
8.0	22.0	55.0
6.0	17.0	43.0
4.0	12.0	30.0
2.0	8.0	16.0
1.0	5.0	9.0
0.50	3.5	5.5
0.25	2.0	4.0
0.10	1.0	3.0
0	0	2.0

rutin and affords a convenient method for its determination. The advantages of fluorimetry over colorimetry are also demonstrated in Table 1, where the readings with different concentrations of rutin are compared. However, the boric-citric reagent itself exhibits some fluorescence which varies with different lots of reagent (6). The effects of moisture, concentration of reagents, time of standing, and other variables have been worked out, and the fluorimetric method is now being applied to the determination of rutin in biological materials, using procedures for extraction similar to those already developed for flavones (4, 6).

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Metabolism Cages

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In laboratories which only occasionally engage in metabolism experiments with rats, it may be difficult to obtain quickly a suitable arrangement of cages. The unit to be described is efficient, easily constructed, and requires few items not usually stocked. The complete unit has gradually evolved over a period of years, and therefore proper credit cannot be given to individuals who have contributed ideas.

The ordinary round cage used in nutrition experiments may be used (Fig. 1). This cage, as shown, has an efficient

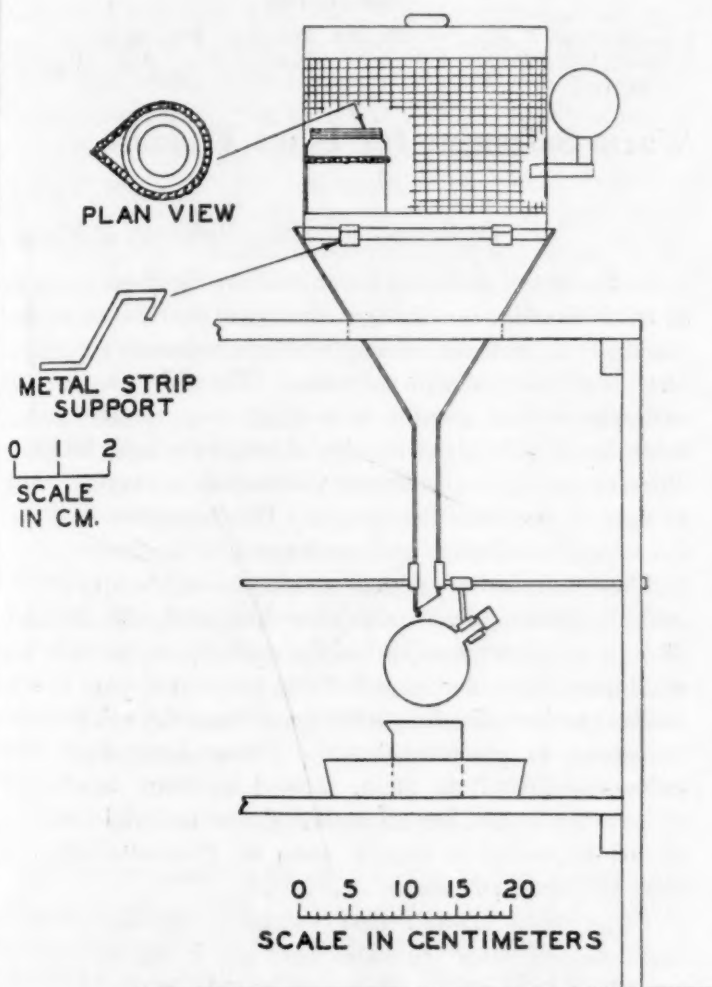


FIG. 1. Arrangement of metabolism cage and stand.

watering device, but the trough, unless cleaned often, tends to accumulate particles of food from the mouth of the rat. If this is objectionable, a drinking tube may be pushed between the wire meshes in the top of the cage, with an inverted bottle outside the cage. The food receptacle is held in place against the cage by a weak spring, which encircles the upper part of the jar. The spring also permits easy removal or replacement of the food jar. For most of our work we use half-pint Mason jars, with Kerr screw caps, a circular opening, 3.5-4 cm. in diameter, being cut through the center of each lid. Although the lids may be dispensed with, their use often helps prevent the animals from throwing out and wasting food.

The cage rests upon four strips of stainless steel or other metal (each about 12 mm. wide), bent on one end to fit over the edge of a funnel, 250 mm. in diameter, and bent up on the other end to form a firm ledge for the cage bottom. These strips may be lifted off for cleaning. The glass funnel fits into a hole in the wooden stand and is gripped by a burette clamp near the lower end of the stem. A 200-ml., round-bottom, short-ring flask (balloon flask) is held by a burette clamp beneath the stem of the funnel. Feces drop between the meshes of the cage and, falling through the stem of the funnel, are deflected by the rounded surface of the flask into a pan or large evaporating dish. Urine passes down the funnel onto the surface of the flask and follows the curve of the flask to the lowest portion, whence it drips into a beaker, or Erlenmeyer flask, placed directly beneath the balloon flask.

The wooden stand, 109 x 63 x 30.5 cm., has three openings, 15.2 cm. in diameter, to support the funnels, and a shelf 19 cm. from the floor to support the receptacles for the urine and feces. A metal rod, 0.9 cm. in diameter and 38 cm. from the floor, runs the entire length of the stand. To this rod are fastened the various burette clamps.

The stand described is designed for three cages, which provides a unit easily moved by one person and conveniently stored.

Sharp Interfacial Precipitin Reactions in Capillary Pipettes¹

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The capillary pipette precipitin technique, which was developed for grouping and typing hemolytic streptococci (2), has proven adaptable to other investigations in which precipitin tests are required. The obvious advantages lie in the great saving of both precipitating serum and bacterial extracts or other antigenic substances. With moderately to strongly reacting reagents, easily detectable precipitates are formed in capillary pipettes having an external diameter of 1.0 ± 0.02 mm., in which the two reagents readily mix. With weaker precipitating sera, or with sera in which prozoning is liable to occur, particularly with those which react with the group-specific carbohydrates of streptococci, it is often necessary to employ larger capillary pipettes (*i.e.* 1.5 ± 0.02 mm.). With such pipettes, however, it is frequently difficult to obtain as clear-cut reactions as occur in small test tubes in which the antigen is carefully layered over the serum so that a sharp ring of precipitate forms at the interface between the two reagents (1). It has long been recognized that ring, or interface precipitin reactions are at times convenient in developing satisfactory precipitin tests. A difficulty encountered in trying to obtain sharp layering in capillary pipettes is the tendency for the two reagents to mix—a tendency roughly proportional to the movement of the interface. This move-

¹ This investigation was carried out under a contract between the Rockefeller Institute for Medical Research and the Commission on Hemolytic Streptococcal Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. Army.

ment is held to a minimum in the larger pipettes by the following modifications:

Pointed capillary pipettes are made from tubing, 1.5 ± 0.2 mm. in external diameter, which is broken into lengths of about 13 cm.² The middle of each length is heated in a narrow flame, such as is made by a fishtail burner or the pilot of a Bunsen burner, and, when melted, drawn to a very fine point by quickly pulling on the two ends of the tubing. When cool, the very finely drawn glass is broken close to the conical tip; thus, two conically pointed pipettes are formed with fine openings about the diameter of a hair. To insure that these openings are patent, they may be inspected with the aid of a hand lens. With practice, three to four such pairs can be drawn at one time. The outer surface of these pipettes is cleaned with paper tissue, the pipettes then being placed in test tubes containing absorbent cotton on which the conical points rest. These containers are capped with unsized paper and then sterilized by dry heat, or by autoclaving followed by drying in an incubator.

For testing, the sterile pointed end of a pipette is dipped into the precipitating serum until a column of serum between 1.0 and 1.5 cm. long has been slowly drawn in by capillary action, then wiped with paper tissue and dipped into a drop of antigen solution until an equal amount has been drawn into the pipette. Air bubbles must not separate serum and antigen solution. This conical end is again wiped and plunged into a lump of plasticine in order to seal the hair-sized opening. The lower end of the pipette is pressed against a strip of plasticine, which has been previously placed on a block of wood, so that the pipette is held in a vertical position without being plunged into the plasticine. If it is mounted in this manner, the fluid in the upper part of the pipette will not be forced out by air pressure which would be developed were the lower open end forced directly into plasticine. The upper end should, however, be inspected to see whether any solution has been forced out, and if this has occurred, it should be wiped off so that no film will be deposited on the pipette.

If this procedure has been followed, the column of fluid will remain at the upper part of the pipette, the surface of which should be perfectly clean.

The above-outlined technique insures the minimal mixing of the underlying serum and overlying antigen solution; hence, there is a narrow zone in which the precipitate forms. When longer columns of serum and antigen are drawn into the capillary pipette, this interzone moves over a proportionally wider range, more mixing of the two reagents occurs, and the zone of precipitate is wider; hence, the reaction may be less intense. The same tendency to mixing of the two reagents exists when the reaction is set up, as previously advocated, in 1.5-cm. pipettes with both ends wide open: rapid mixing of the two reagents occurs, and the reactions may be so indeterminate that they often require confirmation in small test tubes.

Within 5 to 10 minutes a positive reaction is shown by a cloudy white disc of very fine precipitate at the junction of serum and antigen. With weak sera or antigens, a longer time may be required. When testing with streptococcal grouping sera, if the pipettes are placed in the incubator at 37° C. for an hour, weak cross-reactions with sera of other

groups sometimes occur; hence, readings made within 5 to 10 minutes probably indicate more specifically the group to which the streptococci under examination belong. Upon standing, the precipitate formed early may redissolve, or it may clump, fall to the bottom of the serum, and be detectable the following morning after refrigeration.

Errors due to weak reactions may result from excessive mixing of the antigen and serum. Another source of error at times is to allow the test to stand too long before reading, because the precipitate formed early may redissolve. False readings may result from grease marks or other materials on the outer surface of the tubes over the zone of reaction. Antigens and sera should always be clear before preparing the test, since hazy sera, or sediment drawn into the pipettes with the reagents, may lead to false-positive readings.

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Warm Safranin for Plant Tissues

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In the course of recent botanical investigations conducted at the University of Michigan Biological Station, an attempt was made to decrease the length of time necessary for staining plant slide material with safranin. The slides and safranin were placed in a paraffin oven which was thermostatically controlled at 53° C., and a series of tests were made subjecting different materials of different thicknesses to varying lengths of time in the heated safranin. The procedure up to and following the safranin bath conformed to the norm.

The results indicated that completely satisfactory staining could be obtained in 15 minutes as compared with the 24- to 48-hour period necessary when the stain is used at room temperature. The color imparted by the warm stain is quite brilliant and remains fast in the tissue even through prolonged immersion in absolute alcohol. Young *Lycopodium* stem, ordinarily difficult to stain, showed excellent results after 15 minutes in the heated stain. Other material tested included hypocotyl of collard, stem of *Prenanthes alba*, and stem of *Diervilla lonicera*.

Other tests showed that successful staining could be accomplished when the slides were left in the safranin for periods up to 3½ hours. However, material left in for 12 hours was overstained, resulting in a deep purple color which was not readily distinguishable, under low power, from the fast green used as a counterstain.

As the heat induces a rapid and thorough penetration of the dye, the excess stain is not as rapidly removed as under room temperature, and it is desirable to leave the slides in absolute alcohol for a somewhat longer time than is usual. For the same reason, the normal 1- to 2-minute immersion in fast green was increased to 3-10 minutes.

A brief examination of the literature indicates no previous mention of the possibilities of using warm safranin for botanical preparations, although Lee (1) mentions its use for zoological preparations by Griesbach in 1887.

Reference

1. LEE, B. *Microtome's vade-mecum*. (9th ed.) Philadelphia: Blakiston, 1928.

² This capillary tubing is carried in stock by Arthur H. Thomas, Philadelphia.

Book Reviews

The diffraction of X-rays and electrons by free molecules.

M. H. Pirene. Cambridge, Engl.: at the Univ. Press; New York: Macmillan, 1946. Pp. xii + 160. (Illustrated.) \$3.00.

The diffraction of electrons by free molecules has been used so extensively in recent years in the study of interatomic distances that it is both useful and refreshing to have a reminder of other aspects. This volume contains an excellent presentation of the physics of the diffraction of X-rays by free molecules. Although it is based entirely on previously published material, it is useful to have this material brought together in one handy volume. Included is a treatment of coherent and incoherent scattering by a free electron, by an atom, by free molecules, and by gases and liquids where the intermolecular effects are not negligible, as well as the effect of thermal motions and the Fourier method of analyzing diffraction data. The mathematical development tends to be somewhat cursory; too often the function under discussion is stated without its derivation being shown. On the other hand, the physical interpretation of the functions is very thorough, and the comparison with experimental data is especially good in the careful statement of the limitations on interpretation imposed by the assumptions inherent in the theory.

The treatment of electron diffraction is not parallel with that of X-rays. Of the 13 chapters, 10 deal exclusively with X-rays. The theoretical treatment for electrons is very brief, and there is no counterpart to Chapter XII, on the experimental technique of X-ray diffraction, and to Chapter XIII, which lists the molecules studied by X-ray diffraction.

No prospective author of a work describing the technique and analyzing the reliability of the results of electron diffraction need be discouraged by the present book.

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Qualitative analysis by spot tests: inorganic and organic application. (3rd Engl. ed., rev.) Fritz Feigl. (Translated by Ralph E. Oesper.) New York-Amsterdam: Elsevier Publishing Co., 1946. Pp. xvi + 574. (Illustrated.) \$8.00.

This third English edition of Dr. Feigl's well-known book on spot tests is the result of a revision of the previous issue which has been expanded to include "the newer spot reactions, together with details of the pertinent procedure and applications." The two previous English editions were translated by Dr. Janet W. Matthews. A change of translators became unavoidable because of the difficulties of communication between Brazil and England, and because of the decision to have the printing done in the United States. A large part of the translation by Matthews is, however, retained in the text.

New features of the third edition are the sections on "Working Methods and Special Aids in Spot Test Analysis," "Tests for Free Elements," and "Bibliography on the Application of Spot Reactions for Special Scientific or Technical Purposes." The first is taken from the author's *Laboratory manual of spot tests*, published by the Academic Press, Inc., New York City. The general usefulness of this section must be granted, but the

aid of a standard text on microtechnique will be desirable whenever the performance of involved separations should become necessary. The Bibliography contains references to 21 books and 134 articles published in journals. As far as is feasible—and this holds for the whole book—references to *Chemical Abstracts* replace the former references to *Chemisches Zentralblatt*.

A listing of the principal sections may serve to indicate the directions in which the text has expanded: Working Methods and Special Aids (8-40); Tests for Metals (41-190); Tests for Acid Radicals (191-271); Tests for Free Elements (272-281); Systematic Analysis of Mixtures (282-308); Qualitative Organic Analysis (309-424); Application in Tests of Purity, Examination of Technical Materials, and Studies of Minerals (425-488); Bibliography on Applications (489-495); Tabular Summary of the Limits of Identification (496-509); Author Index (511-518); and Subject Index (519-574). In the section on organic analysis, 10 pages are devoted to the detection of elements, 73 to the detection of characteristic groups, and 32 to a description of the identification of specific compounds.

The author has tried to present the entire literature on spot tests in order to give the reader a rapid survey of the whole field, but it is interesting to observe that the stimulating articles of B. L. Clarke and Hermance as well as those of H. Yagoda completely failed to deflect the author from his traditional course. The text has been expanding ever since publication of the first German edition in 1931, but its general trend has remained unchanged.

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Faune de l'Empire française. Paris: Museum d'Histoire Naturelle, 1943-. Vol. 1 + .

French zoologists must accept from their colleagues in other countries not merely congratulations but profound admiration and gratitude that throughout the difficult conditions of war and of their country's occupation they have been able to continue the well-known *Faune française* and to launch a parallel series dealing with the possessions, colonies, and protectorates that form their worldwide, in larger part tropical empire.

The rich collections which have been received from overseas for more than a century and which have accumulated in the Museum d'Histoire Naturelle de Paris, serve as a basis for the enormous research contemplated and well begun in the preparation of this series. The entrepreneurs estimate that in less favorable cases more than half of the fauna of these diverse regions is already known; and in many others, almost the total.

The series was initiated in 1943 by a volume on the Orthoptéroïdes of North Africa, by Lucien Chopard, the one man in the world most capable of writing it. It established a general design for the work and a truly high plane of scientific competence. The plan is that of a complete manual, with introductory pages on morphology and biology. The taxonomic portion includes keys to all groups down to species, and under each of the latter the synonymy, an adequate description, the

distribution (accompanied by precise records) the ethology and the subspecies (if any) are dealt with. Illustrations delineating differentiating characters are numerous, and there are complete illustrations of the principal types, so that from these alone one may gain some idea of the variations of habitus within the fauna.

The last volume to appear, just issued, is Part I of the "Coléoptères carabiques de la région malgache," by René Jeannel. It follows two volumes in the *Faune de France* by the same author, dealing with the same group, in which a long-awaited new classification of these beetles has been presented, based in large measure on research concerning the structure of the male genitalia, which was previously disregarded. In the opinion of the reviewer this represents not merely a revolution but a strong advance in our knowledge of the interrelationships of these beetles (although his judgment would question the necessity of assigning family rank to so many subdivisions). It is a matter of moment to entomologists to see this new classification extended in detail to the Caraboidea (in which term Cicindelidae and Paussidae as well as Carabidae are included) of the Malagasy Subregion. Dr. Jeannel accepts the theories of continental drift of Köppen and Wegener, and his introductory pages on the origin of the Malagasy faunas, as well as his account of the climate, vegetation, and biogeographical divisions, will be of wide interest.

Intermediate between the first and latest volumes to appear have been one on the Bovidae of French Equatorial Africa (from the Congo to the Sudan and Rio de Oro), by Paul Rode; one on the Scarabaeid beetles of Indo-China, by Renaud Paulian (which, along with his treatment of the Scarabaeidae in the *Faune de France*, is no less worthy of note than the volumes on carabid beetles); one on the birds of Reunion, by Jacques Berlioz; and one on the longicorn beetles of North Africa, by Andre Villiers.

The early appearance is promised of a volume completing the Caraboidea of Madagascar, of one by E. Fletiaux on the Coleoptera of the French Antilles, one by A. Villiers on the reduviid Hemiptera of tropical Africa, and of one dealing with the polychaete annelids of New Caledonia, by P. Fauvel. The 23 volumes in preparation form an exciting list: 12 on insects; 2 each on arachnids, molluscs, and fish; and 1 each on mammals, birds, lizards, ctenophores, and corals. Such a series of manuals of tropical faunas has been previously attempted only in the fauna of British India, but that, of course, deals with only a single area.

The Editorial Committee consists of R. Jeannel, J. Millot, Th. Monod, L. Berland, and L. Chopard, both of the latter being secretaries.

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